AA

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 December 2002 (27.12.2002)

PCT

(10) International Publication Number
WO 2002/102973 A3

(51) International Patent Classification[7]: C07K 16/00, A61K 39/395

(21) International Application Number: PCT/IL2002/000495

(22) International Filing Date: 20 June 2002 (20.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/299,187 20 June 2001 (20.06.2001) US

(71) Applicant *(for all designated States except US)*: **PROCHON BIOTECH LTD.** [IL/IL]; P.O. Box 1482, 76114 Rehovot (IL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **YAYON, Avner** [IL/IL]; Moshav Sitria #104, 76834 (IL). **ROM, Eran** [IL/IL]; Gordon Street 43, 76287 Rehovot (IL).

(74) Agent: **WEBB, Cynthia**; Webb & Associates, P.O. Box 2189, 76121 Rehovot (IL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report: 26 February 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2002/102973 A3

(54) Title: ANTIBODIES THAT BLOCK RECEPTOR PROTEIN TYROSINE KINASE ACTIVATION, METHODS OF SCREENING FOR AND USES THEREOF

(57) Abstract: Molecules containing the antigen-binding portion of antibodies that block constitutive and/or ligand-dependent activation of a receptor protein tyrosine kinase, such as fibroblast growth factor receptor 3 (FGFR3), are found through screening methods, where a soluble dimeric form of a receptor protein tyrosine kinase is used as target for screening a library of antibody fragments displayed on the surface of bacteriophage. The molecules of the present invention which block constitutive activation can be administered to treat or inhibit skeletal dysplasia, craniosynostosis disorders, cell proliferative diseases or disorders, or tumor progression associated with the constitutive activation of a receptor protein tyrosine kinase.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL02/00495

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C07K 16/00; A61K 39/395
US CL : 530/387.1, 388.1, 388.22, 388.8, 388.85, 300, 350; 424/130.1, 143.1, 155.1, 156.1.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/387.1, 388.1, 388.22, 388.8, 388.85, 300, 350; 424/130.1, 143.1, 155.1, 156.1.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 6,214,974 B1 (ROSENBLUM et al.) 10 April 2001 (10.04.2001), see entire document, especially column 2, line 44-55.	1, 4-5, 7-8, 11 ---------- 12
Y	US 5,707,632 A (WILLIAMS et al.) 13 January 1998 (13.01.1998), see entire document, especially column 21-23.	1-5, 7-9, 11, 12
X --- Y	US 6,165,464 A (HUDZIAK et al.) 26 December 2000 (26.12.2000), see entire document, especially abstract.	1, 4, 7 ---------- 12
X --- Y	US 6,129,915 A (WELS et al.) 10 October 2000 (10.10.2000), see entire document, especially abstract, column 2, lines 1-5.	1-2, 4, 7 ---------- 12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 18 August 2003 (18.08.2003)	Date of mailing of the international search report 05 DEC 2003
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230	Authorized officer Larry R. Helms Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL02/00495

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claim Nos.: 13-37, 41-85
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-12

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-12, drawn to an antibody.

Group II, claim(s) 38-40, drawn to a method of screening a molecule.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The technical feature linking Groups I-II appears to be an antibody to FGFR. However, Rosenblum et al (US Patent 6,214,974, issued 4/2001) teach such an antibody (see column 2, lines 44-55). Therefore, the technical feature linking the inventions of Groups I-II does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

Continuation of B. FIELDS SEARCHED Item 3:

CAPLUS MEDLINE BIOSIS, WEST, USPATFUL

search terms: FGFR, FGFR3, her2/neu, EGFR, fibroblast growth factor receptor, receptor protein tyrosine kinase, activation.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 December 2002 (27.12.2002)

PCT

(10) International Publication Number
WO 02/102973 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/IL02/00495

(22) International Filing Date: 20 June 2002 (20.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/299,187 20 June 2001 (20.06.2001) US

(71) Applicant *(for all designated States except US)*: **PROCHON BIOTECH LTD.** [IL/IL]; P.O. Box 1482, 76114 Rehovot (IL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **YAYON, Avner** [IL/IL]; Moshav Sitria #104, 76834 (IL). **ROM, Eran** [IL/IL]; Gordon Street 43, 76287 Rehovot (IL).

(74) Agent: **WEBB, Cynthia**; Webb & Associates, P.O. Box 2189, 76121 Rehovot (IL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/102973 A2

(54) Title: ANTIBODIES THAT BLOCK RECEPTOR PROTEIN TYROSINE KINASE ACTIVATION, METHODS OF SCREENING FOR AND USES THEREOF

(57) Abstract: Molecules containing the antigen-binding portion of antibodies that block constitutive and/or ligand-dependent activation of a receptor protein tyrosine kinase, such as fibroblast growth factor receptor 3 (FGFR3), are found through screening methods, where a soluble dimeric form of a receptor protein tyrosine kinase is used as target for screening a library of antibody fragments displayed on the surface of bacteriophage. The molecules of the present invention which block constitutive activation can be administered to treat or inhibit skeletal dysplasia, craniosynostosis disorders, cell proliferative diseases or disorders, or tumor progression associated with the constitutive activation of a receptor protein tyrosine kinase.

ANTIBODIES THAT BLOCK RECEPTOR PROTEIN TYROSINE KINASE ACTIVATION, METHODS OF SCREENING FOR AND USES THEREOF

Field of the Invention

The present invention relates to: immunoglobulins (and functional fragments thereof) useful for blocking activation of receptor protein tyrosine kinases, methods for screening for such immunoglobulins, compositions comprising said immunoglobulins and methods of using the same for treating or inhibiting disease, such as skeletal dysplasia, craniosynostosis disorders, cell proliferative diseases or disorders, or tumor progression.

Background of the Invention

A wide variety of biological processes involves complex cellular communication mechanisms. One of the primary means of continual exchange of information between cells and their internal and external environments is via the secretion and specific binding of peptide growth factors. Growth factors exert pleiotropic effects and play important roles in oncogenesis and the development of multicellular organisms regulating cell growth, differentiation and migration. Many of these factors mediate their effects by binding to specific cell surface receptors. The ligand-activated receptors start an enzymatic signal transduction cascade from the cell membrane to the cell nucleus, resulting in specific gene regulation and diverse cellular responses.

Protein Kinases

One of the key biochemical mechanisms of signal transduction involves the reversible phosphorylation of proteins, which enables regulation of the activity of mature proteins by altering their structure and function.

Protein kinases ("PKs") are enzymes that catalyze the phosphorylation of hydroxy groups on tyrosine, serine and threonine residues of proteins. The consequences of this seemingly simple activity are staggering; cell growth, differentiation and proliferation; e.g., virtually all aspects of cell life in one way or another depend on PK activity. Furthermore, abnormal PK activity has been related to a host of disorders, ranging from relatively non-life threatening diseases such as psoriasis to extremely virulent diseases such as glioblastoma.

The kinases fall largely into two groups, those specific for phosphorylating serine and threonine, and those specific for phosphorylating tyrosine. Some kinases, referred to as "dual specificity" kinases, are able to phosphorylate tyrosine as well as serine/threonine residues. Protein kinases can also be characterized by their location within the cell. Some kinases are transmembrane receptor proteins capable of binding ligands external to the cell membrane. Binding the ligands alters the receptor protein kinase's catalytic activity. Others are non-receptor proteins lacking a transmembrane domain and yet others are ecto-kinases that have a catalytic domain on the extracellular (ecto) portion of a transmembrane protein or which are secreted as soluble extracellular proteins.

Many kinases are involved in regulatory cascades where their substrates may include other kinases whose activities are regulated by their phosphorylation state. Thus, activity of a downstream effector is modulated by phosphorylation resulting from activation of the pathway.

Receptor protein tyrosine kinases (RPTKs) are a subclass of transmembrane-spanning receptors endowed with intrinsic, ligand-stimulatable tyrosine kinase activity. RPTK activity is tightly controlled. When mutated or altered structurally, RPTKs can become potent oncoproteins, causing cellular transformation. In principle, for all RPTKs involved in cancer, oncogenic deregulation results from relief or perturbation of one or several of the auto-control mechanisms that ensure the normal repression of catalytic domains. More than half of the known RPTKs have been repeatedly found in either mutated or overexpressed forms associated with human malignancies (including sporadic cases; Blume-Jensen et al., 2001). RPTK over expression leads to constitutive kinase activation by increasing the concentration of dimers. Examples are Neu/ErbB2 and epidermal growth factor receptor (EGFR), which are often amplified in breast and lung carcinomas and the fibroblast growth factors (FGFR) associated with skeletal and proliferative disorders (Blume-Jensen et al., 2001).

Fibroblast Growth Factors

Normal growth, as well as tissue repair and remodeling, require specific and delicate control of activating growth factors and their receptors. Fibroblast Growth Factors (FGFs) constitute a family of over twenty structurally related polypeptides that are developmentally regulated and expressed in a wide variety of tissues. FGFs stimulate proliferation, cell migration and differentiation and play a major role in skeletal and limb development, wound healing, tissue repair, hematopoiesis, angiogenesis, and tumorigenesis (reviewed in Ornitz and Itoh, 2001).

The biological action of FGFs is mediated by specific cell surface receptors belonging to the RPTK family of protein kinases. These proteins consist of an extracellular ligand binding domain, a single transmembrane domain and an intracellular tyrosine kinase domain which undergoes phosphorylation upon binding of FGF. The FGF receptor (FGFR) extracellular region contains three immunoglobulin-like (Ig-like) loops or domains (D1, D2 and D3), an acidic box, and a heparin binding domain. Five FGFR genes that encode for multiple receptor protein variants have been identified to date.

Another major class of cell surface binding sites includes binding sites for heparan sulfate proteoglycans (HSPG) that are required for high affinity interaction and activation of all members of the FGF family. Tissue-specific expression of heparan sulfate structural variants confer ligand-receptor specificity and activity of FGFs.

FGFR-Related Disease

Recent discoveries show that a growing number of skeletal abnormalities, including achondroplasia, the most common form of human dwarfism, result from mutations in FGFRs. Specific point mutations in different domains of FGFR3 are associated with autosomal dominant human skeletal disorders including hypochondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN) and thanatophoric dysplasia (TD) (Cappellen et al., 1999; Webster et al., 1997; Tavormina et al.,1999). FGFR3 mutations have also been described in two craniosynostosis phenotypes: Muenke coronal craniosynostosis (Bellus et al., 1996; Muenke et al., 1997) and Crouzon syndrome with acanthosis nigricans (Meyers et al., 1995). Crouzon syndrome is associated with specific point mutations in FGFR2 and both familial and sporadic forms of Pfeiffer syndrome are associated with mutations in FGFR1 and FGFR2 (Galvin et al., 1996; Schell et al., 1995). Mutations in FGFRs result in constitutive activation of the mutated receptors and increased receptor protein tyrosine kinase activity, rendering cells and tissue unable to differentiate. Specifically, the achondroplasia mutation results in enhanced stability of the mutated receptor, dissociating receptor activation from down-regulation, leading to restrained chondrocyte maturation and bone growth inhibition (reviewed in Vajo et al., 2000).

There is accumulating evidence for mutations activating FGFR3 in various types of cancer. Constitutively activated FGFR3 in a large proportion of two common epithelial cancers, bladder and cervix, as well as in multiple myeloma, is the first evidence of an oncogenic role for FGFR3 in carcinomas. FGFR3 currently appears to be the most frequently mutated

oncogene in bladder cancer where it is mutated in almost 50% of the cases and in about 70% of cases having recurrent superficial bladder tumors (Cappellen, et al, 1999; van Rhijn, et al, 2001; Billerey, et al, 2001). FGFR3 mutations are seen in 15-20% of multiple myeloma cases where point mutations that cause constitutive activation directly contribute to tumor development and progression (Chesi, et al, 1997; Plowright, et al, 2000, Ronchetti, et al, 2001).

In this context, the consequences of FGFR3 signaling appear to be cell type-specific. In chondrocytes, FGFR3 hyperactivation results in growth inhibition (reviewed in Ornitz, 2001), whereas in the myeloma cell it contributes to tumor progression (Chesi et al., 2001).

In view of the link between RPTK-related cellular activities and a number of human disorders various strategies have been employed to target the receptors and/or their variants for therapy. Some of these have involved biomimetic approaches using large molecules patterned on those involved in the cellular processes, e.g., mutant ligands (US Patent 4,966,849); soluble receptors and antibodies (WO 94/10202, US 6,342,219); RNA ligands (US Patent 5,459,015) and tyrosine kinase inhibitors (WO 94/14808; US Patent 5,330,992).

Antibody therapy

The search for new therapies to treat cancer and other diseases associated with growth factors and their corresponding cell surface receptors has resulted in the development of humanized antibodies capable of inhibiting receptor function. For example, US patents 5,942,602 and 6,365,157 disclose monoclonal antibodies specific for the HER2/neu and VEGF receptors, respectively. US patent 5,840,301 discloses a chimeric, humanized monoclonal antibody that binds to the extracellular domain of VEGF and neutralizes ligand-dependent activation.

There is an unmet need for highly selective molecules capable of blocking aberrant constitutive receptor protein tyrosine kinase activity, in particular FGFR activity, thereby addressing the clinical manifestations associated with the above-mentioned mutations, and modulating various biological functions.

Citation of any document herein is not intended as an admission that such document is pertinent prior art, or considered material to the patentability of any claim of the present application. Any statement as to content or a date of any document is based on the information available to applicant at the time of filing and does not constitute an admission as to the correctness of such a statement.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide molecules which are able to block receptor protein tyrosine kinase (RPTK) activity.

It is an object of the present invention to provide molecules which are able to block fibroblast growth factor receptor (FGFR) activity, and more preferably fibroblast growth factor receptor 3 (FGFR3) activity.

It is another object of the present invention to provide a method to screen for molecules which are able to block said receptor activity.

It is yet another object of the present invention to provide a pharmaceutical composition comprising as an active ingredient a molecule of the invention useful in treating or preventing skeletal and proliferative diseases and disorders.

It is a further object of the present invention to provide a method for inhibiting growth of tumor cells associated with ligand-dependent or constitutive activation of a receptor protein tyrosine kinase, preferably a fibroblast growth factor receptor, and more preferably FGFR3.

It is yet a further object of the present invention to provide a method for treating skeletal disorders associated with ligand-dependent or constitutive activation of a receptor protein tyrosine kinase, preferably a fibroblast growth factor receptor, and more preferably FGFR3.

It is yet a further object of the present invention to provide a method for blocking receptor protein tyrosine kinase activation in the cells of patients in need thereof by treatment with molecules capable of inhibiting receptor protein tyrosine kinase function.

It is yet another object of the present invention to provide a method for inhibiting tumor growth, tumor progression or metastases.

It is still a further object to provide molecules useful for in vivo imaging of diseased states.

It is still a further object of the invention to provide a kit containing molecules of the invention.

These and other objects are met by the invention disclosed herein.

The present invention provides a molecule that contains the antigen-binding portion of an antibody which has a specific affinity for a receptor protein tyrosine kinase and which blocks constitutive activation of a receptor protein tyrosine kinase. The present invention further

provides a molecule that contains the antigen-binding portion of an antibody which has a specific affinity for a receptor protein tyrosine kinase and which blocks ligand-dependent activation of a fibroblast growth factor receptor (FGFR), including FGFR1 and FGFR3.

Certain molecules of the present invention were found to inhibit or block constitutive, or ligand independent, activation of the FGFR3. Generation of inhibitory molecules would be useful for developing medicaments for use in treating or preventing skeletal and proliferative diseases and disorders associated with constitutive activation of receptor protein tyrosine kinases.

Certain mutations in the genes of receptor protein tyrosine kinases result in activation of the receptor in a manner that is independent of the presence of a ligand. Such ligand-independent, or constitutive, receptor protein tyrosine kinase activation results in increased receptor activity. The clinical manifestations of certain mutations are skeletal and proliferative disorders and diseases, including achondroplasia and various cancers.

Furthermore, the present invention is directed to novel molecules comprising an antigen binding domain which binds to a receptor protein tyrosine kinase and blocks constitutive activation of said receptor protein tyrosine kinase. The molecules of the invention maybe antibodies or antigen binding fragments thereof.

A currently preferred embodiment of the present invention provides a molecule which binds to the extracellular domain of a receptor protein tyrosine kinase and blocks constitutive and ligand-dependent activation of the receptor.

A currently more preferred embodiment of the present invention provides a molecule which binds to the extracellular domain of an FGF receptor and blocks constitutive and ligand-dependent activation of the receptor.

A currently most preferred embodiment of the present invention provides a molecule which binds FGFR3 and blocks constitutive and ligand-dependent activation of the receptor, comprising V_L-CDR3 and V_H-CDR3 regions having SEQ ID NO:25 and 24, respectively and the corresponding polynucleotide sequence SEQ ID NO:51 and 50.

A currently most preferred embodiment of the present invention provides a molecule which binds FGFR3 and blocks constitutive and ligand-dependent activation of the receptor, comprising V_L-CDR3 and V_H-CDR3 regions having SEQ ID NO:13 and 12 or SEQ ID NO:9

and 8, respectively and the corresponding polynucleotide sequence SEQ ID NO:35 and 34 or SEQ ID NO: 31 and 30.

Another currently preferred embodiment of the present invention provides a molecule herein denoted MSPRO12 comprising a light chain having SEQ ID NO:94 and a heavy chain having SEQ ID NO:105 and the corresponding polynucleotide sequences having SEQ ID NO:75 and 89, respectively.

Another currently preferred embodiment of the present invention provides a molecule herein denoted MSPRO2 comprising a light chain having SEQ ID NO:92 and a heavy chain having SEQ ID NO:103 and the corresponding polynucleotide sequences having SEQ ID NO:74 and 86,

Another currently most preferred embodiment of the present invention provides a molecule herein denoted MSPRO59 comprising a light chain having SEQ ID NO:102 and a heavy chain having SEQ ID NO:113 and the corresponding polynucleotide sequences having SEQ ID NO:76 and 91, respectively.

According to the principles of the present invention, molecules which bind FGFR and block ligand-dependent receptor activation are provided. These molecules are useful in treating disorders and diseases associated with an FGFR that is activated in a ligand-dependent manner including certain skeletal disorders, hyperproliferative diseases or disorders and non-neoplastic angiogenic pathologic conditions such as neovascular glaucoma, macular degeneration, hemangiomas, angiofibromas, psoriasis and proliferative retinopathy including proliferative diabetic retinopathy.

A currently most preferred embodiment of the present invention provides a molecule which binds FGFR3 and blocks ligand-dependent activation of the receptor, comprising V_H-CDR3 and V_L-CDR3 regions having SEQ ID NO:20 and 21 , respectively and the corresponding polynucleotide sequence SEQ ID NO:44 and 45, respectively.

Other currently preferred embodiments of the present invention provides a molecule which binds FGFR3 and blocks ligand-dependent activation of the receptor, comprising V_H-CDR3 and V_L-CDR3 regions selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:11; SEQ ID NO:14 and SEQ ID NO:15; SEQ ID NO:16 and SEQ ID NO:17; SEQ ID NO:18 and SEQ ID NO:19; SEQ ID NO:20 and SEQ ID NO:21; SEQ ID NO:26 and SEQ ID NO:27 or SEQ ID NO:28 and SEQ ID NO:29 and the corresponding polynucleotide sequences according to table 1B.

Additional currently preferred embodiments of the present invention provide molecules having an antigen binding domain comprising a VL region and a VH region, respectively, selected from the group consisting of respectively, selected from the group consisting of SEQ ID NO: 92 and 103; SEQ ID NO: 93 and 104; SEQ ID NO: 94 and 105; SEQ ID NO:95 and 106; SEQ ID NO: 96 and 107 ; SEQ ID NO: 97 and 108; SEQ ID NO:98 and 109; SEQ ID NO: 99 and 110; SEQ ID NO: 101 and 112; and SEQ ID NO:102 and 113.

A currently preferred embodiment of the present invention provides a molecule comprising V_H-CDR3 and V_L-CDR3 domains having SEQ ID NO:22 and SEQ ID NO:23, which has specific affinity for FGFR1 and which blocks ligand-dependent activation of FGFR1, and the corresponding polynucleotides having SEQ ID NO:46 and SEQ ID NO:47.

A currently preferred embodiment of the present invention provides a molecule comprising domains having SEQ ID NO: 100 and 111, which has specific affinity for FGFR1 and which blocks ligand-dependent activation of FGFR1, and the corresponding polynucleotides having SEQ ID NO:73 and SEQ ID NO:82.

In addition, the present invention also relates to methods for screening for the molecules according to the present invention by using a dimeric form of a receptor protein tyrosine kinase as a target for screening a library of antibody fragments.

According to one currently preferred embodiment the screening method comprises

screening a library of antibody fragments for binding to a dimeric form of a receptor protein tyrosine kinase;

identifying an antibody fragment which binds to the dimeric form of the receptor protein tyrosine kinase as a candidate molecule for blocking constitutive activation of the receptor protein tyrosine kinase; and

determining whether the candidate molecule blocks constitutive or ligand-dependent activation of the receptor protein tyrosine kinase in a cell.

According to another currently preferred embodiment the dimeric form of the RPTK is a soluble extracellular domain of a receptor protein tyrosine kinase. Non-limiting examples of receptor protein tyrosine kinases disclosed in Blume-Jensen et al. (2001) include EGFR/ErbB1, ErbB2/HER2/Neu, ErbB/HER3, ErbB4/HER4, IGF-1R, PDGFR-α, PDGFR-β, CSF-1R, kit/SCFR, Flk2/FH3, Flk1/VEGFR1, Flk1/VEGFR2, Flt4/VEGFR3, FGFR1,

FGFR2/K-SAM, FGFR3, FGFR4, TrkA, TrkC, HGFR, RON, EphA2, EphB2, EphB4, Axl, TIE/TIE1, Tek/TIE2, Ret, ROS, Alk, Ryk, DDR, LTK and MUSK.

By using a dimeric form of the RPTK as bait in the screen, a molecule which would bind to the dimeric form of the receptor has been identified. This presents a novel concept in screening for antibodies or fragments thereof with the capacity to bind to a constitutively activated RPTK such as those associated with various disorders and diseases. It also presents an opportunity to screen for molecules which bind to a heterodimer RPTK. A further aspect of the present invention provides a pharmaceutical composition comprising as an active ingredient a molecule of the present invention useful for preventing or treating skeletal or cartilage diseases or disorders and craniosynostosis disorders associated with constitutive or ligand-dependent activation of a receptor protein tyrosine kinase.

In a currently preferred embodiment the pharmaceutical compositions of the present invention may be used for treating or preventing skeletal disorders associated with aberrant FGFR signaling, including achondroplasia, thanatophoric dysplasia, Apert or Pfeiffer syndrome and related craniosynostosis disorders.

A further aspect of the present invention provides a pharmaceutical composition comprising as an active ingredient a molecule of the present invention useful for preventing or treating cell proliferative diseases or disorders or tumor progression, associated with the constitutive or ligand-dependent activation of a receptor protein tyrosine kinase.

In a currently preferred embodiment the pharmaceutical compositions of the present invention may be used for treating or preventing proliferative diseases associated with aberrant FGFR signaling, including multiple myeloma, transitional cell carcinoma of the bladder, mammary and cervical carcinoma, chronic myeloid leukemia and osteo- or chondrosarcoma.

A further aspect of the invention provides molecules comprising an antigen binding domain which can be conjugated to cytotoxins useful for targeting cells expressing said antigen.

Another currently preferred aspect of the present invention provides molecules comprising an antigen binding domain which can be conjugated to appropriate detectable imaging moiety, useful for in vivo tumor imaging.

A still further aspect of the present invention provides methods for treating or inhibiting the aforementioned diseases and disorders by administering a therapeutically effective amount of

a pharmaceutical composition comprising a molecule of the present invention to a subject in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows hFR3$^{23-374}$TDhis purification by Coommassie stained SDS-PAGE.

Figure 2 shows hFR3$^{23-374}$TDhis binding to heparin and FGF9.

Figure 3 shows the purification of FR3exFc and FR1exFc on SDS-PAGE.

Figure 4 shows the neutralization effect of the hFR3$^{23-374}$TDhis and FR3exFc soluble receptors in a ligand-dependent proliferation assay.

Figure 5 shows the effect of MSPRO Fabs on proliferation of FGFR1 and FGFR3-expressing cells.

Figure 6 shows the effect of MSPRO Fabs on proliferation of FGFR3-expressing cells.

Figures 7A and 7B show the neutralizing activity of several MSPRO Fabs in a proliferation assay using the FDCP-FR3 (C10; Fig. 7A) or the FDCP-FR1 cells (Fig. 7B).

Figure 8 shows the receptor specificity of MSPRO Fabs on RCJ-FR3 cells by Western blot using an anti-P-ERK antibody. Figure 8A shows different MSPRO Fabs while Figure 8B shows a dose response of MSPRO 12, 29 and 13 on RCJ-FR3 cells.

Figures 9A-9D demonstrates the specificity and potency of MS-PRO Fabs by Western blot with anti-P-ERK antibody.

Figure 10 shows a diagrammatic representation of FGFR3 and of FGFR3 truncations (D2-3, D2) and isoforms (IIIb, IIIc). The isoform IIIb differs from IIIc at the carboxy terminus of the IgIII domain as indicated with a dotted line.

Figure 11 shows that the FGFR3 neutralizing Fabs recognize IgII or IgII and III in the extracellular region of FGFR3.

Figure 12 shows that MSPRO29 specifically recognizes the IIIc isoform of FGFR3.

Figure 13 shows the results of a proliferation assay for FDCP-FR3IIIb or FDCP-FR3IIIc cells incubated with increasing dose of the indicated Fabs.

Figure 14 shows iodinated MSPRO29 binding to FGFR3.

Figure 15 shows results of a proliferation assay is a graph wherein iodinated MSPRO29 retained its activity against FGFR3.

Figures 16A-16F show the selective binding of radiolabelled MS-PRO29 to histological of growth plate.

Figure 17 shows a proliferation assay of FDCP-FR3 (C10) and FDCP-FR3ach cells incubated with FGF9 and with increasing doses of the indicated Fabs.

Figure 18B shows that MSPRO12 and MSPRO59 inhibit the ligand independent proliferation of FDCP-FR3ach cells. Fig. 18A shows analysis of the ligand-dependent FDCP-FR3wt cells.

Figure 19 shows the restoration of cell growth in RCS cells by MS-PRO54 and MSPRO59..

Figure 20 represents the growth rate of treated bone with MS-PRO 59.

Figure 21 is a flow chart of the experimental protocol for assessing receptor activation and signaling.

Figure 22 shows ^{125}I labeled MSPRO59 localization to the FDCP-FR3ach derived tumor.

Figure 23 shows the effect of MSPRO59 on inhibiting ligand-independent tumor growth after 24 and 26 days.

Figure 24 shows the effect of MSPRO59 on inhibiting ligand-independent tumor growth.

Figure 25A shows the effect of MSPRO59 on inhibiting ligand-independent tumor growth. Figure 25B shows scFv MSPRO59 blocking the proliferation of FDCP-FR3 (S375C) cells.

Figure 26 shows the effect of MSPRO59 single chain antibody on inhibiting ligand-independent tumor growth.

Figure 27 shows binding of Fab Miniantibodies to FGFR3-Fc and FGFR1-Fc (ELISA).

Figure 28A is an example of a Fab expression vector for use in accordance with the present invention.

Figure 28B is the DNA sequence of the vector according to Figure 28A

Figure 29A is an example of a phage display vector for use in accordance with the present invention.

Figure 29B is the DNA sequence of the vector according to Figure 29A.

Figure 30 depicts the polynucleotide sequences of the VL and VH of MSPRO antibodies of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the discovery that neutralizing antibodies that block ligand-dependent and ligand-independent activation of fibroblast growth factor 3 (FGFR3), a receptor protein tyrosine kinase (RPTK), can be obtained by screening an antibody library against a dimeric form of the extracellular portion of FGFR3. Until the present invention, the present inventors are unaware of any success in obtaining neutralizing antibodies that block constitutive activation of any RPTK including FGFR or ligand-dependent FGFR activation.

The term "receptor protein tyrosine kinase" or "RPTK" as used herein and in the claims refers to a subclass of transmembrane-spanning receptors endowed with intrinsic, ligand-stimulatable tyrosine kinase activity. RPTKs comprise a large family of spatially and temporally regulated proteins that control many different aspects of growth and development. When mutated or altered structurally, RPTKs can undergo deregulation and become activated in a ligand-independent, or constitutive, manner. In certain cases they become potent oncoproteins, causing cellular transformation.

As used herein and in the claims the term "fibroblast growth factor receptor" or "FGFR" denotes a receptor specific for FGF which is necessary for transducing the signal exerted by FGF to the cell interior, typically comprising an extracellular ligand-binding domain, a single transmembrane helix, and a cytoplasmic domain having tyrosine kinase activity. The FGFR extracellular domain consists of three immunoglobulin-like (Ig-like) domains (D1, D2 and D3), a heparin binding domain and an acidic box. Alternative splicing of the FGF receptor mRNAs generates different variants of the receptors.

Molecules, including antibodies and fragments thereof, comprising an antigen binding domain to a receptor protein tyrosine kinase are highly necessary for the treatment of various pathological conditions.

In the past, the laboratory of the present inventors encountered difficulties in raising neutralizing antibodies against FGFR3. When mice were immunized with the soluble monomeric FGFR3 receptor, by the time the antibody titers begins to increase, the mice died. The experiments performed in the laboratory of the present inventors that failed to obtain anti-FGFR3 neutralizing antibodies in mice are presented in the Examples.

By using a soluble dimeric form of the extracellular domain of the FGFR3 receptor to screen for antibodies, e.g., Fabs, that bind from a phage display antibody library, the present inventors were able to overcome a problem in the prior art for which there was yet no solution and to obtain numerous high affinity ($K_D < 10$ nM) antibodies (Fabs) that bind FGFR3 and interfere with ligand binding, thereby blocking ligand-dependent activation of FGFR3 signaling. Very surprisingly, from among the group of Fabs that block ligand-dependent activation, Fab antibodies which also block ligand-independent (constitutive) activation of FGFR3 by blocking signaling caused by constitutive dimerization of FGFR3 were identified. To the best of the present inventors' knowledge, the Fab antibodies obtained which block constitutive activation of FGFR3 are the first antibodies against any receptor protein tyrosine kinase that blocks constitutive, ligand-independent activation/signaling.

Trastuzamab, an anti-human epidermal growth factor receptor 2 (HER2) antibody, was the first humanized monoclonal antibody approved for therapeutic use. Its mode of action appears to be manifold, including HER2 down regulation, prevention of heterodimer formation, prevention of HER2 cleavage and others (Baselga and Albanell, 2001). US patents 5, 677171; 5772997; 6165464 and 6,399,063 disclose the anti-HER2 invention but neither teach nor suggest that the antibody blocks ligand-independent receptor activation.

One aspect of the present invention is directed to neutralizing antibodies and more generally to a molecule that includes the antigen binding portion of an antibody which blocks ligand-dependent activation and constitutive, ligand-independent activation of a receptor protein tyrosine kinase, preferably an FGFR and more preferably FGFR3.

Another aspect of the present invention is directed to molecules comprising an antigen binding domain which blocks ligand-dependent activation of an FGFR, more preferably FGFR3.

The molecule having the antigen-binding portion of an antibody according to the present invention can be used in a method for blocking the ligand-dependent activation and/or ligand independent (constitutive) activation of FGFR3. Preferred embodiments of such antibodies/molecules, obtained from an antibody library designated as HuCAL® (Human Combinatorial Antibody Library) clone, is presented in Table 1 with the unique VH-CDR3 and VL-CDR3 sequences given.

In addition to sequencing of the clones, a series o biochemical assays were performed to determine affinity and specificity of the molecules to the respective receptors.

TABLE 1A

HuCAL® Clone	VH-CDR3 Sequence	VL-CDR3 equence	Framework
MSPRO2	DFLGYEFDY (SEQ ID NO: 8)	QSYDYSADY (SEQ IDNO: 9)	VH1B_L3
MSPRO11	YYGSSLYHYVFGGFIDY (SEQ ID NO: 10)	QSHHFYE (SEQ ID NO: 11)	VH1B_L2
MSPRO12	YHSWYEMGYYGSTVGYMFD (SEQ ID NO: 12)	QSYDFDFA (SEQ ID NO: 13)	VH2_L3
MSPRO21	DNWFKPFSDV (SEQ ID NO: 14)	QQYDSIPY (SEQ ID NO: 15)	VH1A_k4
MSPRO24	VNHWTYTFDY (SEQ ID NO: 16)	QQMSNYPD (SEQ ID NO: 17)	VH1A_k3
MSPRO26	GYWYAYFTYINYGYFDN (SEQ ID NO: 18)	QSYDNNSDV (SEQ ID NO: 19)	VH1B_L2
MSPRO28	GGGWVSHGYYYLFDL (SEQ ID NO: 26)	FQYGSIPP (SEQ ID NO: 27)	VH1A_k1
MSPRO29	TWQYSYFYYLDGGYYFDI (SEQ ID NO: 20)	QQTNNAPV (SEQ ID NO:21)	VH1B_k3
MSPRO54	NMAYTNYQYVNMPHFDY (SEQ ID NO: 22)	QSYDYFKL (SEQ ID NO:23)	VH1B_L3
MSPRO55	SMNSTMYWYLRRVLFDH (SEQ ID NO: 28)	QSYDMYMYI (SEQ ID NO: 29)	VH1B_L2
MSPRO59	SYYPDFDY (SEQ ID NO:24)	QSYDGPDLW (SEQ ID NO:25)	VH6_L3

VH refers to the variable heavy chain, VL refers to the variable light chain; L refers to the lambda light chain and k refers to the kappa light chain

Table 1B lists the corresponding polynucleotide sequences of the CDR domains.

TABLE 1B

HuCAL® Clone	VH-CDR3 polynucleotide sequence	VL-CDR3 olynucleotide Sequence
MSPRO2	GATTTTCTTGGTTATGAGTTTGATTAT (SEQ ID NO:30)	CAGAGCTATGAC TATTCTGCT GAT TAT (SEQ ID NO:31)
MSPRO11	TATTATGGTTCTTCTCTTTATCATTATGTTT TTGGTGGTTTTATTGATTAT (SEQ ID NO:32)	CAGTCTCATCAT TTTTATGAG (SEQ ID NO:33)
MSPRO12	TATCATTCTTGGTATGAGATGGGTTATTAT GGTTCTACTGTTGGTTATATGTTTGATTAT (SEQ ID NO:34)	CAGAGCTATGAC TTTGATTTT GCT (SEQ ID NO:35)
MSPRO21	GATAATTGGTTTAAGCCTTTTTCTGATGTT (SEQ ID NO:36)	CAGCAGTATGAT TCTATTCCT TAT (SEQ ID NO:37)
MSPRO24	GTTAATCATTGGACTTATACTTTTGATTAT (SEQ ID NO:38)	CAGCAGATGTCT AATTATCCTGAT (SEQ ID NO:39)
MSPRO26	GGTTATTGGTATGCTTATTTTACTTAT ATTAATTATGGTTATTTT GATAAT (SEQ ID NO:40)	CAGAGCTATGAC AATAATTCTGAT GTT (SEQ ID NO:41)
MSPRO28	GGTGGTGGTTGGGTTTCTCATGGTTATTAT TATCTTTTTGATCTT (SEQ ID NO:42)	TTTCAGTATGGT TCTATTCCT CCT (SEQ ID NO: 43)
MSPRO29	ACTTGGCAGTATTCTTATTTTTATTAT CTTGATGGTGGTTATTATTTTGATATT (SEQ ID NO:44)	CAGCAGACTAAT AATGCTCCTGTT (SEQ ID NO:45)
MSPRO54	AATATGGCTTATACTAATTATCAGTATGTT AATATGCCTCATTTTGATTAT (SEQ ID NO:46)	CAGAGCTATGAC TATTTTAAGCTT (SEQ ID NO:47)
MSPRO55	TCTATGAATTCTACTATGTATTGGTATCTT CGTCGTGTTCTTTTTGAT CAT (SEQ ID NO:48)	CAGAGCTATGAC ATGTATAATTAT ATT (SEQ ID NO:49)
MSPRO59	TCTTATTAT CCTGATTTT GATTAT (SEQ ID NO:50)	CAGAGCTATGAC GGTCCTGATCTT TGG (SEQ ID NO:51)

Figure 30 provides the polynucleotide sequences of the preferred embodiments of the invention. The amino acid sequence of the VH and VL domains of the currently preferred embodiments of the present invention are presented below.

MS-Pro-2-VL(SEQ ID NO:92)

```
  1   DIELTQPPSV SVAPGQTARI SCSGDALGDK YASWYQQKPG QAPVLVIYDD
 51   SDRPSGIPER FSGSNSGNTA TLTISGTQAE DEADYYCQSY DYSADYVFGG
101   GTKLTVLGQ
```

MS-Pro-11-VL(SEQ ID NO:93)

```
  1   DIALTQPASV SGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLMI
 51   YDVSNRPSGV SNRFSGSKSG NTASLTISGL QAEDEADYYC QSHHFYEVFG
101   GGTKLTVLGQ
```

MS-PRO-12-VL (SEQ ID NO:94)

```
  1   DIELTQPPSV SVAPGQTARI SCSGDALGDK YASWYQQKPG QAPVLVIYDD
 51   SDRPSGIPER FSGSNSGNTA TLTISGTQAE DEADYYCQSY DFDFAVFGGG
101   TKLTVLGQ
```

MS-Pro-21-VL(SEQ ID NO:95)

```
  1   DIVMTQSPDS LAVSLGERAT INCRSSQSVL YSSNNKNYLA WYQQKPGQPP
 51   KLLIYWASTR ESGVPDRFSG SGSGTDFTLT ISSLQAEDVA VYYCQQYDSI
101   PYTFGQGTKV EIKRT
```

MS-Pro-24-VL(SEQ ID NO:96)

```
  1   DIVLTQSPAT LSLSPGERAT LSCRASQSVS SSYLAWYQQK PGQAPRLLIY
 51   GASSRATGVP ARFSGSGSGT DFTLTISSLE PEDFATYYCQ QMSNYPDTFG
101   QGTKVEIKRT
```

MS-Pro-26-VL (SEQ ID NO:97)

```
  1   DIALTQPASV SGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLMI
 51   YDVSNRPSGV SNRFSGSKSG NTASLTISGL QAEDEADYYC QSYDNNSDVV
101   FGGGTKLTVL GQ
```

MS-Pro-28-VL (SEQ ID NO:98)

```
  1   DIQMTQSPSS LSASVGDRVT ITCRASQGIS SYLAWYQQKP GKAPKLLIYA
 51   ASSLQSGVPS RFSGSGSGTD FTLTISSLQP EDFAVYYCFQ YGSIPPTFGQ
101   GTKVEIKRT
```

MS-Pro-29-VL (SEQ ID NO:99)

1 DIVLTQSPAT LSLSPGERAT LSCRASQSVS SSYLAWYQQK PGQAPRLLIY

51 GASSRATGVP ARFSGSGSGT DFTLTISSLE PEDFATYYCQ QTNNAPVTFG

101 QGTKVEIKRT

MS-Pro-54-VL (SEQ ID NO:100)

1 DIELTQPPSV SVAPGQTARI SCSGDALGDK YASWYQQKPG QAPVLVIYDD

51 SDRPSGIPER FSGSNSGNTA TLTISGTQAE DEADYYCQSY DYFKLVFGGG

101 TKLTVLGQ

MS-Pro-55-VL (SEQ ID NO:101)

1 DIALTQPASV SGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLMI

51 YDVSNRPSGV SNRFSGSKSG NTASLTISGL QAEDEADYYC QSYDMYNYIV

101 FGGGTKLTVL GQ

MS-Pro-59-VL (SEQ ID NO:102)

1 DIELTQPPSV SVAPGQTARI SCSGDALGDK YASWYQQKPG QAPVLVIYDD

51 SDRPSGIPER FSGSNSGNTA TLTISGTQAE DEADYYCQSY DGPDLWVFGG

101 GTKLTVLGQ

MS-Pro-2-VH (SEQ ID NO:103)

1 QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMHWVRQA PGQGLEWMGW

51 INPNSGGTNY AQKFQGRVTM TRDTSISTAY MELSSLRSED TAVYYCARDF

101 LGYEFDYWGQ GTLVTVSS

MS-Pro-11-VH (SEQ ID NO:104)

1 QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMHWVRQA PGQGLEWMGW

51 INPNSGGTNY AQKFQGRVTM TRDTSISTAY MELSSLRSED TAVYYCARYY

101 GSSLYHYVFG GFIDYWGQGT LVTVSS

MS-Pro-12-VH(SEQ ID NO:105)

1 QVQLKESGPA LVKPTQTLTL TCTFSGFSLS TSGVGVGWIR QPPGKALEWL

51 ALIDWDDDKY YSTSLKTRLT ISKDTSKNQV VLTMTNMDPV DTATYYCARY

101 HSWYEMGYYG STVGYMFDYW GQGTLVTVSS

MS-Pro-21-VH (SEQ ID NO:106)

1 QVQLVQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGG

51 IIPIFGTANY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARDN

101 WFKPFSDVWG QGTLVTVSS

MS-Pro-24-VH (SEQ ID NO:107)

1 QVQLVQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGG

51 IIPIFGTANY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARVN

101 HWTYTFDYWG QGTLVTVSS

MS-Pro-26-VH(SEQ ID NO:108)

1 QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMHWVRQA PGQGLEWMGW

51 INPNSGGTNY AQKFQGRVTM TRDTSISTAY MELSSLRSED TAVYYCARGY

101 WYAYFTYINY GYFDNWGQGT LVTVSS

MS-Pro-28-VH(SEQ ID NO:109)

1 QVQLVQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGG

51 IIPIFGTANY AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARGG

101 GWVSHGYYYL FDLWGQGTLV TVSS

MS-Pro-29-VH(SEQ ID NO:110)

1 QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMHWVRQA PGQGLEWMGW

51 INPNSGGTNY AQKFQGRVTM TRDTSISTAY MELSSLRSED TAVYYCARTW

101 QYSYFYYLDG GYYFDIWGQG TLVTVSS

MS-Pro-54-VH(SEQ ID NO:111)

1 QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMHWVRQA PGQGLEWMGW

51 INPNSGGTNY AQKFQGRVTM TRDTSISTAY MELSSLRSED TAVYYCARNM

101 AYTNYQYVNM PHFDYWGQGT LVTVSS

MS-Pro-55-VH(SEQ ID NO:112)

1 QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMHWVRQA PGQGLEWMGW

51 INPNSGGTNY AQKFQGRVTM TRDTSISTAY MELSSLRSED TAVYYCARSM

101 NSTMYWYLRR VLFDHWGQGT LVTVSS

MS-Pro-59-VH (SEQ ID NO:113)

1 QVQLQQSGPG LVKPSQTLSL TCAISGDSVS SNSAAWNWIR QSPGRGLEWL

51 GRTYYRSKWY NDYAVSVKSR ITINPDTSKN QFSLQLNSVT PEDTAVYYCA

101 RSYYPDFDYW GQGTLVTVSS

In addition to sequencing of the clones, a series of biochemical assays were performed to determine affinity and specificity of the molecules to the respective receptors. Table 1C lists the affinity of the respective molecules to FGFR3 and FGFR1 as measures by Biacore and/or FACS. In a binding assay to FGFR3-expressing cells the IC_{50} of the molecules was

calculated (Example 6). Domain specificity was determined as described in Example 9. The ligand-independent inhibition of FGFR3 (neutralizing activity) was determined as described in Example 11. Finally, the molecules were synthesized in a number of different formats including Fab, miniantibody (Fab-dHLX), IgG1, IgG4, IgG3 and as single chain Fv (scFv).

Table 1C

Clone	Affinity to FGFR3 (BIAcore)	Affinity to FGFR3 (FACS)	Affinity to FGFR1	Koff (s^{-1})	IC50 FR3 (FGF9)	Domain Specificity	Ligand independent inhibition of FGFR3	Available formats
MSPRO59	1.5nM	<1nM	-	7.1x10e-4	19 nM	2	+.	Fab, Fab-dHLX IgG1, IgG4, mIgG3, scFv
MSPRO2	37nM	43 nM	-	2x10e-2	360 nM	2	~	Fab(+/- tags), Fab-dHLX, IgG1, IgG4,
MSPRO12	14nM	6.5 nM	-	2.3x10e-3	58 nM	2	+	Fab (+/- tag), Fab-dHLX, IgG1, IgG4, scFv
MSPRO11	4	4 nM	108	4 x 10e-4	220 nM	3		Fab. Fab-dHLX
MSPRO21	9 nM		-	3.6x10e-3	50 nM	3c		Fab, IgG1, Fab-dHLX
MSPRO24	10 nM		-	5.4x10e-3	70 nM	3c		Fab, IgG1
MSPRO26	4 nM	1.4		5 x 10e-4	70 nM	3		Fab, Fab-dHLX
MSPRO28	9 nM	0.3 nM	160 nM	4 x10e-3	50 nM	3		Fab
MSPRO29	6 nM	<1nM	29 nM	1.4x10e-3	20nM	3c	-	Fab (+/- tag), IgG1, IgG4, Fab-dHLX, scFv
MSPRO54	3.7nM		2.5nM	2x10e-3	45nM	3c		Fab, IgG1
MSPRO55	2.9nM		-	7.4x10e-4	34nM	3c		Fab

BiaCore results for certain molecules

In Table 1D the numbers are the IC_{50}s of the dimeric dHLX format of certain binders (molecule with antigen binding site) in the FDCP-FGFR3 proliferation assay performed with FGF9. The numbers in parentheses are the IC_{50} of the monomeric Fabs in the same assay. Table 1E presents the KD value for certain MSPRO molecules in miniantibody form, as determined in a Biacore.

Table 1D

binder	IC_{50}
MSPRO2	61 nM (360)
MSPRO12	26 nM (58)
MSPRO21	20 nM (50)
MSPRO26	8 nM (70)

Table 1E

K_D determination for certain molecules

Clone	BIAcore K_D [nM]	Number of measurements
MS-Pro-2-dHLX-MH	4.3 (37)	1
MS-Pro-11-dHLX-MH	0.7 (4)	1
MS-Pro-12-dHLX-MH	1.2 (14)	1
MS-Pro-21-dHLX-MH	2.2 (4.1)	1
MS-Pro-24-dHLX-MH	2 (10)	1
MS-Pro-26-dHLX-MH	2 (4)	1
MS-Pro-28-dHLX-MH	1.6 (9)	1

The preferred, but non-limiting, embodiments of molecules according to the present invention that block constitutive (ligand-independent) activation of FGFR3 are referred to herein MSPRO2, MSPRO12 and MSPRO59 comprising VH-CDR3 and VL-CDR3 domains having SEQ ID Nos: 8 and 9; 12 and 13; and 24 and 25, respectively. The preferred, but non-limiting, embodiments of molecules according to the present invention that block ligand-dependent activation of FGFR3 are referred to herein MSPRO11, MSPRO21, MSPRO24, MSPRO26, MSPRO29, and MSPRO54 comprising VH-CDR3 and VL-CDR3 domains

having SEQ ID Nos: 10 and 11; 14 and 15; 16 and 17, 18 and 19; 21 and 22; 23 and 24, respectively. Preferably, an antibody or a molecule of the present invention has an affinity (K_D) for binding a soluble dimeric form of FGFR3 of less than about 50 nM, preferably less than about 30 nM and more preferably less than about 10 nM, as determined by the BIAcore chip assay for affinity, by a FACS-Scatchard analysis or other methods known in the art.

While the specific discovery of an antibody/molecule that blocks constitutive activation was made with respect to FGFR3 using a soluble dimeric form of FGFR3 to screen a phage display antibody library, it is believed that for all, or almost all receptor protein tyrosine kinases, antibodies/molecules that block constitutive activation can be similarly obtained using a soluble dimeric form of a corresponding extracellular domain of a receptor protein tyrosine kinase. Non-limiting examples of receptor protein tyrosine kinases disclosed in Blume-Jensen et al. (2001) include EGFR/ErbB1, ErbB2/HER2/Neu, ErbB/HER3, ErbB4/HER4, IGF-1R, PDGFR-α, PDGFR-β, CSF-1R, kit/SCFR, Flk2/FH3, Flk1/VEGFR1, Flk1/VEGFR2, Flt4/VEGFR3, FGFR1, FGFR2/K-SAM, FGFR3, FGFR4, TrkA, TrkC, HGFR, RON, EphA2, EphB2, EphB4, Axl, TIE/TIE1, Tek/TIE2, Ret, ROSAlk,Ryk, DDR, LTK and MUSK.

Furthermore, antibodies/molecules that block ligand-dependent or ligand independent activation of heterodimer receptor protein tyrosine kinases are intended to be included in the scope of the invention. Heterodimerization is well documented for members of the EGFR subfamily of receptor protein tyrosine kinases and others. Non-limiting examples include EGFR/PDGFRβ, Flt1/KDR and EGFR/ErbB2 heterodimers. EGFR and PDGFRβ heterodimers have been identified as a mechanism for PDGF signal transduction in rat vascular smooth muscle cells (Saito et al., 2001) and Flt-1/KDR heterodimers are required for vinculin assembly in focal adhesions in response to VEGF signaling (Sato et al., 2000).

The present invention is also directed to a molecule having the antigen-binding portion of an antibody which binds to a dimeric form of an extracellular portion of a receptor protein tyrosine kinase (RPTK), such as a FGFR, and blocks the ligand-independent (constitutive) activation and/or ligand-dependent activation of the RPTK.

Antibodies

Antibodies, or immunoglobulins, comprise two heavy chains linked together by disulfide bonds and two light chains, each light chain being linked to a respective heavy chain by disulfide bonds in a "Y" shaped configuration. Proteolytic digestion of an antibody yields Fv

(Fragment variable and Fc (fragment crystalline) domains. The antigen binding domains, Fab', include regions where the polypeptide sequence varies. The term $F(ab')_2$ represents two Fab' arms linked together by disulfide bonds. The central axis of the antibody is termed the Fc fragment. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains (CH). Each light chain has a variable domain (VL) at one end and a constant domain (CL) at its other end, the light chain variable domain being aligned with the variable domain of the heavy chain and the light chain constant domain being aligned with the first constant domain of the heavy chain (CH1).

The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the light and heavy chains have the same general structure and each domain comprises four framework regions, whose sequences are relatively conserved, joined by three hypervariable domains known as complementarity determining regions (CDR1-3). These domains contribute specificity and affinity of the antigen binding site.

The isotype of the heavy chain (gamma, alpha, delta, epsilon or mu) determines immunoglobulin class (IgG, IgA, IgD, IgE or IgM, respectively). The light chain is either of two isotypes (kappa,κ or lambda,λ) found in all antibody classes.

It should be understood that when the terms "antibody" or "antibodies" are used, this is intended to include intact antibodies, such as polyclonal antibodies or monoclonal antibodies (mAbs), as well as proteolytic fragments thereof such as the Fab or $F(ab')_2$ fragments. Further included within the scope of the invention are chimeric antibodies; human and humanized antibodies; recombinant and engineered antibodies, and fragments thereof. Furthermore, the DNA encoding the variable region of the antibody can be inserted into the DNA encoding other antibodies to produce chimeric antibodies (see, for example, US patent 4,816,567). Single chain antibodies fall within the scope of the present invention. Single chain antibodies can be single chain composite polypeptides having antigen binding capabilities and comprising amino acid sequences homologous or analogous to the variable regions of an immunoglobulin light and heavy chain (linked VH-VL or single chain Fv (ScFv)). Both V_H and V_L may copy natural monoclonal antibody sequences or one or both of the chains may comprise a CDR-FR construct of the type described in US patent 5,091,513, the entire contents of which are hereby incorporated herein by reference. The separate polypeptides analogous to the variable regions of the light and heavy chains are held together by a polypeptide linker. Methods of production of such single chain antibodies, particularly

where the DNA encoding the polypeptide structures of the V_H and V_L chains are known, may be accomplished in accordance with the methods described, for example, in US patents 4,946,778, 5,091,513 and 5,096,815, the entire contents of each of which are hereby incorporated herein by reference.

Additionally, CDR grafting may be performed to alter certain properties of the antibody molecule including affinity or specificity. A non-limiting example of CDR grafting is disclosed in US patent 5,225,539.

A "molecule having the antigen-binding portion of an antibody" as used herein is intended to include not only intact immunoglobulin molecules of any isotype and generated by any animal cell line or microorganism, but also the antigen-binding reactive fraction thereof, including, but not limited to, the Fab fragment, the Fab' fragment, the $F(ab')_2$ fragment, the variable portion of the heavy and/or light chains thereof, Fab miniantibodies (see WO 93/15210, US patent application 08/256,790, WO 96/13583, US patent application 08/817,788, WO 96/37621, US patent application 08/999,554, the entire contents of which are incorporated herein by reference) and chimeric or single-chain antibodies incorporating such reactive fraction, as well as any other type of molecule or cell in which such antibody reactive fraction has been physically inserted, such as a chimeric T-cell receptor or a T-cell having such a receptor, or molecules developed to deliver therapeutic moieties by means of a portion of the molecule containing such a reactive fraction. Such molecules may be provided by any known technique, including, but not limited to, enzymatic cleavage, peptide synthesis or recombinant techniques.

The term “Fc” as used herein is meant as that portion of an immunoglobulin molecule (Fragment crystallizable) that mediates phagocytosis, triggers inflammation and targets Ig to particular tissues; the Fc portion is also important in complement activation.

In one embodiment of the invention, a chimera comprising a fusion of the extracellular domain of the RPTK and an immunoglobulin constant domain can be constructed useful for assaying for ligands for the receptor and for screening for antibodies and fragments thereof

The "extracellular domain" when used herein refers the polypeptide sequence of the RPTKs disclosed herein which are normally positioned to the outside of the cell. The extracellular domain encompasses polypeptide sequences in which part of or all of the adjacent (C-terminal) hydrophobic transmembrane and intracellular sequences of the mature RPTK have

been deleted. Thus, the extracellular domain-containing polypeptide can comprise the extracellular domain and a part of the transmembrane domain. Alternatively, in the preferred embodiment, the polypeptide comprises only the extracellular domain of the RPTK. The truncated extracellular domain is generally soluble. The skilled practitioner can readily determine the extracellular and transmembrane domains of a RPTK by aligning the RPTK of interest with known RPTK amino acid sequences for which these domains have been delineated. Alternatively, the hydrophobic transmembrane domain can be readily delineated based on a hydrophobicity plot of the polypeptide sequence. The extracellular domain is N-terminal to the transmembrane domain.

The term "epitope" is meant to refer to that portion of any molecule capable of being bound by an antibody or a fragment thereof which can also be recognized by that antibody. Epitopes or antigenic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics.

An "antigen" is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen may have one or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

A "neutralizing antibody" as used herein refers to a molecule having an antigen binding site to a specific receptor capable of reducing or inhibiting (blocking) activity or signaling through a receptor, as determined by *in vivo* or *in vitro* assays, as per the specification.

A monoclonal antibody (mAb) is a substantially homogeneous population of antibodies to a specific antigen. MAbs may be obtained by methods known to those skilled in the art. See, for example Kohler et al (1975); US patent 4,376,110; Ausubel et al (1987-1999); Harlow et al (1988); and Colligan et al (1993), the contents of which references are incorporated entirely herein by reference. The mAbs of the present invention may be of any immunoglobulin class including IgG, IgM, IgE, IgA, and any subclass thereof. A hybridoma producing an mAb may be cultivated *in vitro* or *in vivo*. High titers of mAbs can be obtained in *in vivo* production where cells from the individual hybridomas are injected intraperitoneally into pristine-primed Balb/c mice to produce ascites fluid containing high

concentrations of the desired mAbs. MAbs of isotype IgM or IgG may be purified from such ascites fluids, or from culture supernatants, using column chromatography methods well known to those of skill in the art.

Chimeric antibodies are molecules, the different portions of which are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region. Antibodies which have variable region framework residues substantially from human antibody (termed an acceptor antibody) and complementarity determining regions substantially from a mouse antibody (termed a donor antibody) are also referred to as humanized antibodies. Chimeric antibodies are primarily used to reduce immunogenicity in application and to increase yields in production, for example, where murine mAbs have higher yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric mAbs are used. Chimeric antibodies and methods for their production are known in the art (Better et al, 1988; Cabilly et al, 1984; Harlow et al, 1988; Liu et al, 1987; Morrison et al, 1984; Boulianne et al, 1984; Neuberger et al, 1985; Sahagan et al , 1986; Sun et al, 1987; Cabilly et al; European Patent Applications 125023, 171496, 173494, 184187, 173494, PCT patent applications WO 86/01533, WO 97/02671, WO 90/07861, WO 92/22653 and US patents 5,693,762, 5,693,761, 5,585,089, 5,530,101 and 5,225,539). These references are hereby incorporated by reference.

Besides the conventional method of raising antibodies *in vivo*, antibodies can be generated *in vitro* using phage display technology. Such a production of recombinant antibodies is much faster compared to conventional antibody production and they can be generated against an enormous number of antigens. In contrast, in the conventional method, many antigens prove to be non-immunogenic or extremely toxic, and therefore cannot be used to generate antibodies in animals. Moreover, affinity maturation (i.e., increasing the affinity and specificity) of recombinant antibodies is very simple and relatively fast. Finally, large numbers of different antibodies against a specific antigen can be generated in one selection procedure. To generate recombinant monoclonal antibodies one can use various methods all based on phage display libraries to generate a large pool of antibodies with different antigen recognition sites. Such a library can be made in several ways: One can generate a synthetic repertoire by cloning synthetic CDR3 regions in a pool of heavy chain germline genes and thus generating a large antibody repertoire, from which recombinant antibody fragments with

various specificities can be selected. One can use the lymphocyte pool of humans as starting material for the construction of an antibody library. It is possible to construct naive repertoires of human IgM antibodies and thus create a human library of large diversity. This method has been widely used successfully to select a large number of antibodies against different antigens. Protocols for bacteriophage library construction and selection of recombinant antibodies are provided in the well-known reference text Current Protocols in Immunology, Colligan et al (Eds.), John Wiley & Sons, Inc. (1992-2000), Chapter 17, Section 17.1.

Another aspect of the present invention is directed to a method for screening for the antibody or molecule of the present invention by screening a library of antibody fragments displayed on the surface of bacteriophage, such as disclosed in the Example herein and described in WO 97/08320, US Patent 6,300,064 , and Knappik et al. (2000), for binding to a soluble dimeric form of a receptor protein tyrosine kinase. An antibody fragment which binds to the soluble dimeric form of the RPTK used for screening is identified as a candidate molecule for blocking ligand-dependent activation and/or constitutive activation of the RPTK in a cell. Preferably the RPTK of which a soluble dimeric form is used in the screening method is a fibroblast growth factor receptor (FGFR), and most preferably FGFR3.

As a first screening method, the soluble dimeric form of a receptor tyrosine kinase can be constructed and prepared in a number of different ways. For instance, the extracellular domain of a RPTK joined to Fc and expressed as a fusion polypeptide that dimerizes naturally by means of the Fc portion of the RPTK-Fc fusion. Other suitable types of constructs of FGFR3, serving as guidance for other RPTKs, are disclosed in the Examples presented herein.

The assays for determining binding of antibody fragments to FGFR3, binding affinities, inhibition of cell proliferation, etc., are also described in the Examples herein below.

The term "cell proliferation" refers to the rate at which a group of cells divides. The number of cells growing in a vessel can be quantified by a person skilled in the art when that person visually counts the number of cells in a defined volume using a common light microscope. Alternatively, cell proliferation rates can be quantified by laboratory apparati that optically or conductively measure the density of cells in an appropriate medium.

A second screen for antibody fragments as candidate molecules can be done using cells having very high over expression of the RPTK, such as for instance RCJ-M15 cells

overexpressing mutant (achondroplasia) FGFR3. In cells expressing very high levels of receptor some ligand-independent activation occurs even without the presence of a mutation, such as a constitutive activation mutation. It is believed that RPTK overexpression forces RPTKs to dimerize and signal even in the absence of ligand. These cells have monomeric receptors as well as dimeric receptors present on their cell surface. Using this type of cell, one of skill in the art would be able to identify all different kinds of antibodies, i.e., blocking ligand-dependent activation, blocking constitutive activation, blocking activation and binding only to monomeric form, etc.

A third screen can be carried out on a cell line expressing a RPTK carrying a mutation, such as the FDCP-FR3ach line expressing the FGFR3 achondroplasia mutation. The receptors of this line become constitutively active, e.g. are able to signal in the absence of a ligand as determined by ERK (MAPK) phosphorylation, a downstream effector.

A further aspect of the present invention relates to a method for treating or inhibiting a skeletal dysplasia or craniosynostosis disorder associated with constitutive activation of a RPTK which involves administering the molecule of the present invention to a subject need thereof. Non-limiting examples of skeletal dysplasias include achondroplasia, thanatophoric dysplasia (TDI or TDII), hypochondroplasia, and severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN) dysplasia. Non-limiting examples of craniosynostosis disorder are Muenke coronal craniosynostosis and Crouzon syndrome with acanthosis nigricans. The symptoms and etiology of these diseases and disorders are reviewed in Vajo et al. (Vajo et al, 2000).

The present invention also provides for a method for treating or inhibiting a cell proliferative disease or disorder associated with the action of an abnormal constitutively activated RPTK, for example. tumor formation, primary tumors, tumor progression or tumor metastasis. A molecule containing the antigen binding portion of an antibody that blocks constitutive activation of a RPTK is administered to a subject in need thereof to treat or inhibit such a cell proliferative disease or disorder.

The terms “treating or inhibiting a proliferative disease or disorder” or “treating or inhibiting a tumor” are used herein and in the claims to encompass tumor formation, primary tumors, tumor progression or tumor metastasis.

Tumor formation or tumor growth are intended to encompass solid and non-solid tumors. Solid tumors include mammary, ovarian, prostate, colon, cervical, gastric, esophageal,

papillary thyroid, pancreatic, bladder, colorectal, melanoma, small-cell lung and non-small-cell lung cancers, granulose cell carcinoma, transitional cell carcinoma, vascular tumors, all types of sarcomas, e.g. osteosarcoma, chondrosarcoma, Kaposi's sarcoma, myosarcoma, hemangiosarcoma, and glioblastomas.

Non-solid tumors include for example hematopoietic malignancies such as all types of leukemia, e.g. chronic myelogenous leukemia (CML), acute myelogenous leukemia (AML), mast cell leukemia, chronic lymphocytic leukemia (CLL) and acute lymphocytic leukemia (ALL), lymphomas, and multiple myeloma (MM).

Tumor progression is the phenomenon whereby cancers become more aggressive with time. Progression can occur in the course of continuous growth, or when a tumor recurs after treatment and includes progression of transitional cell carcinoma, osteo or chondrosarcoma, multiple myeloma, and mammary carcinoma (one of the known RPTKs involved in mammary carcinoma is ErbB3).

The role of the FGFR3 in tumor progression associated with transitional cell carcinoma and multiple myeloma has recently been elucidated (Cappellen, et al, 1999; Chesi, et al, 2001)

In another aspect of the present invention, molecules which bind FGFR, more preferably FGFR3, and block ligand-dependent receptor activation are provided. These molecules are useful in treating hyperproliferative diseases or disorders and non-neoplastic angiogenic pathologic conditions such as neovascular glaucoma, proliferative retinopathy including proliferative diabetic retinopathy, macular degeneration, hemangiomas, angiofibromas, and psoriasis. The role of FGFs and their receptors in neo- and hypervascularization has been well documented (Frank, 1997; Gerwins et al, 2000)

In another aspect of the present invention, the pharmaceutical compositions according to the present invention is similar to those used for passive immunization of humans with other antibodies. Typically, the molecules of the present invention comprising the antigen binding portion of an antibody will be suspended in a sterile saline solution for therapeutic uses. The pharmaceutical compositions may alternatively be formulated to control release of active ingredient (molecule comprising the antigen binding portion of an antibody) or to prolong its presence in a patient's system. Numerous suitable drug delivery systems are known and include, e.g., implantable drug release systems, hydrogels, hydroxymethylcellulose, microcapsules, liposomes, microemulsions, microspheres, and the like. Controlled release preparations can be prepared through the use of polymers to complex or adsorb the molecule

according to the present invention. For example, biocompatible polymers include matrices of poly(ethylene-co-vinyl acetate) and matrices of a polyanhydride copolymer of a stearic acid dimer and sebaric acid (Sherwood et al, 1992). The rate of release molecule according to the present invention, i.e., of an antibody or antibody fragment, from such a matrix depends upon the molecular weight of the molecule, the amount of the molecule within the matrix, and the size of dispersed particles (Saltzman et al., 1989 and Sherwood et al., 1992). Other solid dosage forms are described in (Ansel et al., 1990 and Gennaro, 1990).

The pharmaceutical composition of this invention may be administered by any suitable means, such as orally, intranasally, subcutaneously, intramuscularly, intravenously, intra-arterially, intralesionally or parenterally. Ordinarily, intravenous (i.v.) or parenteral administration will be preferred.

It will be apparent to those of ordinary skill in the art that the therapeutically effective amount of the molecule according to the present invention will depend, *inter alia* upon the administration schedule, the unit dose of molecule administered, whether the molecule is administered in combination with other therapeutic agents, the immune status and health of the patient, the therapeutic activity of the molecule administered and the judgment of the treating physician. As used herein, a "therapeutically effective amount" refers to the amount of a molecule required to alleviate one or more symptoms associated with a disorder being treated over a period of time.

Although an appropriate dosage of a molecule of the invention varies depending on the administration route, age, body weight, sex, or conditions of the patient, and should be determined by the physician in the end, in the case of oral administration, the daily dosage can generally be between about 0.01-200 mg, preferably about 0.01-10 mg, more preferably about 0.1-10 mg, per kg body weight. In the case of parenteral administration, the daily dosage can generally be between about 0.001-100 mg, preferably about 0.001-1 mg, more preferably about 0.01-1 mg, per kg body weight. The daily dosage can be administered, for example in regimens typical of 1-4 individual administration daily. Various considerations in arriving at an effective amount are described, e.g., in Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th ed., Pergamon Press, 1990; and Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pa., 1990.

The molecule of the present invention as an active ingredient is dissolved, dispersed or admixed in an excipient that is pharmaceutically acceptable and compatible with the active

ingredient as is well known. Suitable excipients are, for example, water, saline, phosphate buffered saline (PBS), dextrose, glycerol, ethanol, or the like and combinations thereof. Other suitable carriers are well-known to those in the art. (See, for example, Ansel et al., 1990 and Gennaro, 1990). In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents.

Combination therapy

The combined treatment of one or more of the molecules of the invention with an anti-neoplastic or anti-chemotherapeutic drug such as doxorubicin, cisplatin or taxol provides a more efficient treatment for inhibiting the growth of tumor cells than the use of the molecule by itself. In one embodiment, the pharmaceutical composition comprises the antibody and carrier with an anti-chemotherapeutic drug.

The present invention also provides for a nucleic acid molecule, which contains a nucleotide sequence encoding the molecule having the antigen binding portion of an antibody that blocks ligand-dependent activation and/or constitutive activation of a receptor protein tyrosine kinase such as FGFR3, and a host cell transformed with this nucleic acid molecule. Furthermore, also within the scope of the present invention is a nucleic acid molecule containing a nucleotide sequence having at least 90% sequence identity, preferably about 95%, and more preferably about 97% identity to the above encoding nucleotide sequence as would well understood by those of skill in the art.

The invention also provides nucleic acids that hybridize under high stringency conditions to polynucleotides having SEQ ID NOs: 8 through 29 and SEQ ID NOs: 62, 64-65, 67, 69-71, 73-76 78-80, 82-87, 89, 90-91 or the complement thereof. As used herein, highly stringent conditions are those which are tolerant of up to about 5-20% sequence divergence, preferably about 5-10%. Without limitation, examples of highly stringent (-10°C below the calculated Tm of the hybrid) conditions use a wash solution of 0.1 X SSC (standard saline citrate) and 0.5% SDS at the appropriate Ti below the calculated Tm of the hybrid. The ultimate stringency of the conditions is primarily due to the washing conditions, particularly if the hybridization conditions used are those which allow less stable hybrids to form along with stable hybrids. The wash conditions at higher stringency then remove the less stable hybrids. A common hybridization condition that can be used with the highly stringent to moderately stringent wash conditions described above is hybridization in a solution of 6 X SSC (or 6 X SSPE), 5 X Denhardt's reagent, 0.5% SDS, 100 μg/ml denatured, fragmented salmon sperm

DNA at an appropriate incubation temperature Ti. *See generally* Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2d edition, Cold Spring Harbor Press (1989)) for suitable high stringency conditions.

Stringency conditions are a function of the temperature used in the hybridization experiment and washes, the molarity of the monovalent cations in the hybridization solution and in the wash solution(s) and the percentage of formamide in the hybridization solution. In general, sensitivity by hybridization with a probe is affected by the amount and specific activity of the probe, the amount of the target nucleic acid, the detectability of the label, the rate of hybridization, and the duration of the hybridization. The hybridization rate is maximized at a Ti (incubation temperature) of 20-25°C below Tm for DNA:DNA hybrids and 10-15°C below Tm for DNA:RNA hybrids. It is also maximized by an ionic strength of about 1.5M Na^+. The rate is directly proportional to duplex length and inversely proportional to the degree of mismatching.

Specificity in hybridization, however, is a function of the difference in stability between the desired hybrid and "background" hybrids. Hybrid stability is a function of duplex length, base composition, ionic strength, mismatching, and destabilizing agents (if any).

The Tm of a perfect hybrid may be estimated for DNA:DNA hybrids using the equation of Meinkoth et al (1984), as

$$Tm = 81.5°C + 16.6 (\log M) + 0.41 (\%GC) - 0.61 (\% \text{ form}) - 500/L$$

and for DNA:RNA hybrids, as

$$Tm = 79.8°C + 18.5 (\log M) + 0.58 (\%GC) - 11.8 (\%GC)^2 - 0.56(\% \text{ form}) - 820/L$$

where M, molarity of monovalent cations, 0.01-0.4 M NaCl,

%GC, percentage of G and C nucleotides in DNA, 30%-75%,

% form, percentage formamide in hybridization solution, and

L, length hybrid in base pairs.

Tm is reduced by 0.5-1.5°C (an average of 1°C can be used for ease of calculation) for each 1% mismatching.

The Tm may also be determined experimentally. As increasing length of the hybrid (L) in the above equations increases the Tm and enhances stability, the full-length rat gene sequence can be used as the probe.

Filter hybridization is typically carried out at 68°C, and at high ionic strength (e.g., 5 - 6 X SSC), which is non-stringent, and followed by one or more washes of increasing stringency, the last one being of the ultimately desired high stringency. The equations for Tm can be used to estimate the appropriate Ti for the final wash, or the Tm of the perfect duplex can be determined experimentally and Ti then adjusted accordingly.

The present invention also relates to a vector comprising the nucleic acid molecule of the present invention. The vector of the present invention may be, e.g., a plasmid, cosmid, virus, bacteriophage or another vector used e.g. conventionally in genetic engineering, and may comprise further genes such as marker genes which allow for the selection of said vector in a suitable host cell and under suitable conditions.

Furthermore, the vector of the present invention may, in addition to the nucleic acid sequences of the invention, comprise expression control elements, allowing proper expression of the coding regions in suitable hosts. Such control elements are known to the artisan and may include a promoter, a splice cassette, translation initiation codon, translation and insertion site for introducing an insert into the vector.

Preferably, the nucleic acid molecule of the invention is operatively linked to said expression control sequences allowing expression in eukaryotic or prokaryotic cells.

Control elements ensuring expression in eukaryotic or prokaryotic cells are well known to those skilled in the art. As mentioned herein above, they usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript.

Methods for construction of nucleic acid molecules according to the present invention, for construction of vectors comprising said nucleic acid molecules, for introduction of said vectors into appropriately chosen host cells, for causing or achieving the expression are well-known in the art (see, e.g., Sambrook et al., 1989; Ausubel et al., 1994).

The invention also provides for conservative amino acid variants of the molecules of the invention. Variants according to the invention also may be made that conserve the overall molecular structure of the encoded proteins. Given the properties of the individual amino acids comprising the disclosed protein products, some rational substitutions will be recognized by the skilled worker. Amino acid substitutions, *i.e.* "conservative substitutions," may be made, for instance, on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved.

For example: (a) nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; (b) polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; (c) positively charged (basic) amino acids include arginine, lysine, and histidine; and (d) negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Substitutions typically may be made within groups (a)-(d). In addition, glycine and proline may be substituted for one another based on their ability to disrupt α-helices. Similarly, certain amino acids, such as alanine, cysteine, leucine, methionine, glutamic acid, glutamine, histidine and lysine are more commonly found in α helices, while valine, isoleucine, phenylalanine, tyrosine, tryptophan and threonine are more commonly found in β-pleated sheets. Glycine, serine, aspartic acid, asparagine, and proline are commonly found in turns. Some preferred substitutions may be made among the following groups: (i) S and T; (ii) P and G; and (iii) A, V, L and 1. Given the known genetic code, and recombinant and synthetic DNA techniques, the skilled scientist readily can construct DNAs encoding the conservative amino acid variants.

As used herein, "sequence identity" between two polypeptide sequences indicates the percentage of amino acids that are identical between the sequences. "Sequence similarity" indicates the percentage of amino acids that either are identical or that represent conservative amino acid substitutions.

Conjugates

One embodiment of the present invention provides molecules of the present invention conjugated to cytotoxins. The cytotoxic moiety of the antibody may be a cytotoxic drug or an enzymatically active toxin or bacterial or plant origin, or an enzymatically active fragment of such a toxin including, but not limited to, diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, curcin, crotin, saponin, gelonin, mitogellin, restrictocin, phenomycin, and enomycin. In another embodiment, the molecules of the present invention are conjugate to small molecule anti-cancer drugs. Conjugates of the antibody and such cytotoxic moieties are made using a variety of bifunctional protein coupling agents. Examples of such reagents include SPDP, IT, bifunctional derivatives of imidoesters such a dimethyl adipimidate HCl, active esters such as disuccinimidyl suberate, aldehydes such as glutaraldehyde, bis-azido compounds such as bis-

(p-azidobenzoyl) hexanediamine, bis-diazonium derivatives, dissocyanates and bis-active fluorine compounds. The lysing portion of a toxin may be joined to the Fab fragment of the antibodies.

Additionally, the molecules of the present invention can also be detected *in vivo* by imaging, for example imaging of cells which have undergone tumor progression or have metastasized. Antibody labels or markers for *in vivo* imaging of RPTKs include those detectable by X-radiography, NMR, PET, or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody.

A specific antibody or antibody portion which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{111}In, ^{99}Tc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for a disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moieties needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries. The labeled antibody or antibody portion will then preferentially accumulate at the location of cells which contain a specific RPTK. In vivo tumor imaging is described in Burchiel et al., (1982

The methods and compositions described herein may be performed, for example, by utilizing pre-packaged diagnostic test kits comprising in one or more containers (i) at least one immunoglobulin of the invention and (ii) a reagent suitable for detecting the presence of said immunoglobulin when bound to its target. A kit may be conveniently used, e.g., in clinical settings or in home settings, to diagnose patients exhibiting a disease (e.g., skeletal dysplasia, craniosynostosis disorders, cell proliferative diseases or disorders, or tumor progression), and to screen and identify those individuals exhibiting a predisposition to such disorders. A composition of the invention also may be used in conjunction with a reagent suitable for detecting the presence of said immunoglobulin when bound to its target, as well as instructions for use, to carry out one or more methods of the invention.

Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention.

EXAMPLES

An important approach to control FGFR3 activity is the generation of reagents that block receptor signaling. Without wishing to be bound by theory, molecules which bind the extracellular domain of the receptor may inhibit the receptor by competing with FGF or heparin binding or, alternatively, by preventing receptor dimerization. Additionally, binding to the extracellular domain may accelerate receptor internalization and turnover. Humanized antibodies are expected to have inhibitory/neutralizing action and are of particular interest since they are considered to be valuable for therapeutic applications, avoiding the human anti-mouse antibody response frequently observed with rodent antibodies. The experiments in which the neutralizing antibodies are screened, identified and obtained using fully synthetic human antibody libraries (for discovering highly specific binders against a wide variety of antigens) and FGFR3 extracellular domain are described below.

Example 1: Attempt to generate anti-FGFR3 antibodies

One hundred micrograms of soluble FGFR3 in complete Freund's Adjuvant were injected into Balb/c 3T3 naive mice (9 animals). Two repeated injections of 20 micrograms were performed at week intervals. 10 days after the second booster injection, blood was drawn from animals and serum was tested for the presence of polyclonal antibodies both by monitoring for binding to the receptor as well as for neutralizing activity at a dilution of 1:50. No significant neutralizing activity was observed in the tested serum (20% at most in some animals). A prefusion injection of 20 micrograms of soluble receptor was administered 1-2 days later but all the mice harboring some activity of neutralizing Ab died. The experiment was repeated twice with the same results.

Example 2: Generation of the FGFR3 Antigens

Two dimeric forms of the extracellular domain of the human FGFR3 were prepared for use as antigen. One was a histidine-tagged domain with a Serine 371 to Cysteine (S371C) substitution (thanatophoric dysplasia (TD) mutation) to facilitate dimerization and the second one an Fc fusion. The S371C variant was shown to bind heparin and FGF9 coated plates and

to inhibit FGF9-dependent FDCP-FR3 proliferation. The Fc fusion was similarly effective in binding assays demonstrating its potential as an inhibitor of FGFR function and as a target for selecting FGFR3 inhibitory molecules. Both soluble receptors were employed to select neutralizing human recombinant antibodies.

The two variants of the FGFR3 extracellular domain were prepared as follows:

1. A construct containing the extracellular portion of FGFR3 with a thanatophoric dysplasia (TD) mutation to facilitate dimer formation conjugated to a His-tag (histidine tag) was generated. A bluescript plasmid comprising the human FGFR3 gene (pBS-hFGFR3) was used as template for PCR with the following primers:

5'-ACGTGCTAGC TGAGTCCTTG GGGACGGAGC AG (SEQ ID NO:2).

5'-ACGTCTCGAG TTAATGGTGA TGGTGATGGT GTGCATACAC **ACA**GCCCGCC TCGTC (SEQ ID NO:3),

wherein the Ser 371 Cys (S371C) substitution is bold and underlined.

The nucleotide sequence encoding the extracellular domain of FGFR3 with the TD substitution is denoted herein SEQ ID NO:7:

TGAGTCCTTG GGGACGGAGC AGCGCGTCGT GGGGCGAGCG GCAGAAGTCC CGGGCCCAGA 60
GCCCGGCCAG CAGGAGCAGT TGGTCTTCGG CAGCGGGGAT GCTGTGGAGC TGAGCTGTCC 120
CCCGCCCGGG GGTGGTCCCA TGGGGCCCAC TGTCTGGGTC AAGGATGGCA CAGGGCTGGT 180
GCCCTCGGAG CGTGTCCTGG TGGGGCCCCA GCGGCTGCAG GTGCTGAATG CCTCCCACGA 240
GGACTCCGGG GCCTACAGCT GCCGGCAGCG GCTCACGCAG CGCGTACTGT GCCACTTCAG 300
TGTGCGGGTG ACAGACGCTC CATCCTCGGG AGATGACGAA GACGGGGAGG ACGAGGCTGA 360
GGACACAGGT GTGGACACAG GGGCCCCTTA CTGGACACGG CCCGAGCGGA TGGACAAGAA 420
GCTGCTGGCC GTGCCGGCCG CCAACACCGT CCGCTTCCGC TGCCCAGCCG CTGGCAACCC 480
CACTCCCTCC ATCTCCTGGC TGAAGAACGG CAGGGAGTTC CGCGGCGAGC ACCGCATTGG 540
AGGCATCAAG CTGCGGCATC AGCAGTGGAG CCTGGTCATG GAAAGCGTGG TGCCCTCGGA 600
CCGCGGCAAC TACACCTGCG TCGTGGAGAA CAAGTTTGGC AGCATCCGGC AGACGTACAC 660
GCTGGACGTG CTGGAGCGCT CCCCGCACCG GCCCATCCTG CAGGCGGGGC TGCCGGCCAA 720
CCAGACGGCG GTGCTGGGCA GCGACGTGGA GTTCCACTGC AAGGTGTACA GTGACGCACA 780
GCCCCACATC CAGTGGCTCA AGCACGTGGA GGTGAACGGC AGCAAGGTGG GCCCGGACGG 840
CACACCCTAC GTTACCGTGC TCAAGACGGC GGGCGCTAAC ACCACCGACA AGGAGCTAGA 900
GGTTCTCTCC TTGCACAACG TCACCTTTGA GGACGCCGGG GAGTACACCT GCCTGGCGGG 960
CAATTCTATT GGGTTTTCTC ATCACTCTGC GTGGCTGGTG GTGCTGCCAG CCGAGGAGGA 1020
GCTGGTGGAG GCTGACGAGG CGGGCTGTGT GTATGCACAC CATCACCATC ACCATTAA 1078

The PCR fragment was digested with XhoI and ligated into pBlueScript digested with EcoRV and XhoI. The resulting plasmid, pBsFR3$^{23-374}$Tdhis, was cleaved with NdeI and

XhoI and the DNA fragment encoding the extracellular domain of FGFR3 was ligated into the same restriction sites in pCEP-Pu/Ac7 (Yamaguchi et al., 1999; Kohfeldt et al., 1997), generating the pCEP-hFR3$^{23\text{-}374}$TDhis plasmid construct.

To express this FGFR3 variant, 293E cells (EBNA virus transfected 293 cells) were transfected with the aforementioned plasmid, pCEP-hFR3$^{23\text{-}374}$TDhis, clones were identified and grown. Cell supernatants analyzed by Western blot with anti-His antibody demonstrated high expression of the soluble receptor. Supernatants from large scale preparations were then subjected to batch affinity purification with Ni-NTA beads and the tagged soluble receptor was eluted by a step gradient ranging from 20 mM to 500 mM imidazol. A sample from each eluate was loaded onto a 7.5% SDS-PAGE and stained with GelCode (Pierce). In parallel Western blot analysis was performed and analyzed with anti-His antibodies. SDS-PAGE (Fig. 1) and immunoblot (not shown) analyses demonstrated peak amounts of purified extracellular FGFR3 in the 2nd (2) 50 mM imidazol fraction. About 0.5 mg of pure protein was obtained following this single step purification. In Figure 1, T=total protein, D= dialysed protein, U= unbound fraction.

To assess whether hFR3$^{23\text{-}374}$TDhis (hFR3-TDhis) retained the ability to associate with heparin and heparin-FGF complex, heparin coated wells were incubated with purified (2, 4 or 10 μg, labeled as FR3 2, FR3 4 or FR3 10, respectively in Fig. 2) or unpurified (FR3 sup) hFR3$^{23\text{-}374}$TDhis with (checkered bar) or without FGF9 (200ng/well, hatched bar). The binding of hFR3$^{23\text{-}374}$TDhis to each well was determined with anti-His antibody. Mock supernatant (M sup), PBS and unpurified mouse FR3AP (FGFR3-alkaline phosphatase, labeled as FRAP sup) were included as controls. This demonstrated that, like what was reported for the wild-type receptor, hFR3$^{23\text{-}374}$TDhis binds to heparin and that this interaction is augmented by the presence of FGF9 (Fig. 2). Finally, it was demonstrated that hFR3$^{23\text{-}374}$TDhis inhibits FDCP-FR3 FGF-dependent proliferation in a dose dependent manner. hFR3$^{23\text{-}374}$TDhis had no inhibitory effect on proliferation when FDCP-FR3 cells were grown in the presence of IL-3. Taken together, hFR3$^{23\text{-}374}$TDhis proved to be a good candidate as a target antigen for screening for FGFR3 neutralizing antibodies.

2. The extracellular domain of FGFR3 and FGFR1 were prepared as Fc fusions (FR3exFc and FR1exFc). The amino acid sequence of FGFR3 (NCBI access no: NP_000133) is denoted herein SEQ ID NO:1.

1 MGAPACALAL CVAVAIVAGA SSESLGTEQR VVGRAAEVPG PEPGQQEQLV FGSGDAVELS

```
61 CPPPGGGPMG PTVWVKDGTG LVPSERVLVG PQRLQVLNAS HEDSGAYSCR QRLTQRVLCH
121 FSVRVTDAPS SGDDEDGEDE AEDTGVDTGA PYWTRPERMD KKLLAVPAAN TVRFRCPAAG
181 NPTPSISWLK NGREFRGEHR IGGIKLRHQQ WSLVMESVVP SDRGNYTCVV ENKFGSIRQT
241 YTLDVLERSP HRPILQAGLP ANQTAVLGSD VEFHCKVYSD AQPHIQWLKH VEVNGSKVGP
301 DGTPYVTVLK TAGANTTDKE LEVLSLHNVT FEDAGEYTCL AGNSIGFSHH SAWLVVLPAE
361 EELVEADEAG SVYAGILSYG VGFFLFILVV AAVTLCRLRS PPKKGLGSPT VHKISRFPLK
421 RQVSLESNAS MSSNTPLVRI ARLSSGEGPT LANVSELELP ADPKWELSRA RLTLGKPLGE
481 GCFGQVVMAE AIGIDKDRAA KPVTVAVKML KDDATDKDLS DLVSEMEMMK MIGKHKNIIN
541 LLGACTQGGP LYVLVEYAAK GNLREFLRAR RPPGLDYSFD TCKPPEEQLT FKDLVSCAYQ
601 VARGMEYLAS QKCIHRDLAA RNVLVTEDNV MKIADFGLAR DVHNLDYYKK TTNGRLPVKW
661 MAPEALFDRV YTHQSDVWSF GVLLWEIFTL GGSPYPGIPV EELFKLLKEG HRMDKPANCT
721 HDLYMIMREC WHAAPSQRPT FKQLVEDLDR VLTVTSTDEY LDLSAPFEQY SPGGQDTPSS
781 SSSGDDSVFA HDLLPPAPPS SGGSRT
```

To construct the FR3exFc fusion, a nucleotide sequence (SEQ ID NO:4) encoding the extracellular domain of FGFR3 was PCR amplified to contain terminal KpnI and BamHI restriction sites for insertion into the KpnI and BamHI sites of pCXFc (SEQ ID NO:5). This insertion positions the extracellular domain of FGFR3 to be expressed as a fusion with the Fc amino acid sequence (SEQ ID NO:6).

SEQ ID NO:4:

```
GCGCGCTGCC TGAGGACGCC GCGGCCCCCG CCCCCGCCAT GGGCGCCCCT GCCTGCGCCC 60
TCGCGCTCTG CGTGGCCGTG GCCATCGTGG CCGGCGCCTC CTCGGAGTCC TTGGGGACGG 120
AGCAGCGCGT CGTGGGGCGA GCGGCAGAAG TCCCGGGCCC AGAGCCCGGC CAGCAGGAGC 180
AGTTGGTCTT CGGCAGCGGG GATGCTGTGG AGCTGAGCTG TCCCCCGCCC GGGGGTGGTC 240
CCATGGGGCC CACTGTCTGG GTCAAGGATG GCACAGGGCT GGTGCCCTCG GAGCGTGTCC 300
TGGTGGGGCC CCAGCGGCTG CAGGTGCTGA ATGCCTCCCA CGAGGACTCC GGGGCCTACA 360
GCTGCCGGCA GCGGCTCACG CAGCGCGTAC TGTGCCACTT CAGTGTGCGG GTGACAGACG 420
CTCCATCCTC GGGAGATGAC GAAGACGGGG AGGACGAGGC TGAGGACACA GGTGTGGACA 480
CAGGGGCCCC TTACTGGACA CGGCCCGAGC GGATGGACAA GAAGCTGCTG GCCGTGCCGG 540
CCGCCAACAC CGTCCGCTTC CGCTGCCCAG CCGCTGGCAA CCCCACTCCC TCCATCTCCT 600
GGCTGAAGAA CGGCAGGGAG TTCCGCGGCG AGCACCGCAT TGGAGGCATC AAGCTGCGGC 660
ATCAGCAGTG GAGCCTGGTC ATGGAAAGCG TGGTGCCCTC GGACCGCGGC AACTACACCT 720
GCGTCGTGGA GAACAAGTTT GGCAGCATCC GGCAGACGTA CACGCTGGAC GTGCTGGAGC 780
GCTCCCCGCA CCGGCCCATC CTGCAGGCGG GGCTGCCGGC CAACCAGACG GCGGTGCTGG 840
GCAGCGACGT GGAGTTCCAC TGCAAGGTGT ACAGTGACGC ACAGCCCCAC ATCCAGTGGC 900
TCAAGCACGT GGAGGTGAAC GGCAGCAAGG TGGGCCCGGA CGGCACACCC TACGTTACCG 960
TGCTCAAGAC GGCGGGCGCT AACACCACCG ACAAGGAGCT AGAGGTTCTC TCCTTGCACA 1020
ACGTCACCTT TGAGGACGCC GGGGAGTACA CCTGCCTGGC GGGCAATTCT ATTGGGTTTT 1080
CTCATCACTC TGCGTGGCTG GTGGTGCTGC CAGCCGAGGA GGAGCTGGTG GAGGCTGACG 1140
```

AGGCGGG 1147

SEQ ID NO:5:

```
GACGGATCGG GAGATCTCCC GATCCCCTAT GGTCGACTCT CAGTACAATC TGCTCTGATG    60
CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT GGAGGTCGCT GAGTAGTGCG   120
CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC   180
TTAGGGTTAG GCGTTTTGCG CTGCTTCGCG ATGTACGGGC CAGATATACG CGTTGACATT   240
GATTATTGAC TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA   300
TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC   360
CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA GGGACTTTCC   420
ATTGACGTCA ATGGGTGGAC TATTTACGGT AAACTGCCCA CTTGGCAGTA CATCAAGTGT   480
ATCATATGCC AAGTACGCCC CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT   540
ATGCCCAGTA CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA   600
TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG   660
ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG TTTTGGCACC   720
AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG   780
GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA   840
CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC   900
GTTTAAACTT AAGCTTGGTA CCGAGCTCGG ATCCCCGTCG TGCATCTATC GAAGGTCGTG   960

GA GAT CCC GAG GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA      1007
   ASP PRO GLU GLU PRO LYS SER CYS ASP LYS THR HIS THR CYS PRO 15

CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC     1055
PRO CYS PRO ALA PRO GLU LEU LEU GLY GLY PRO SER VAL PHE LEU PHE 31

CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC     1103
PRO PRO LYS PRO LYS ASP THR LEU MET ILE SER ARG THR PRO GLU VAL 47

ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC     1151
THR CYS VAL VAL VAL ASP VAL SER HIS GLU ASP PRO GLU VAL LYS PHE 63

AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG     1199
ASN TRP TYR VAL ASP GLY VAL GLU VAL HIS ASN ALA LYS THR LYS PRO 79

CGG GAG GAG CAG TAC AAC AGC ACG TAC CGG GTG GTC AGC GTC CTC ACC     1247
ARG GLU GLU GLN TYR ASN SER THR TYR ARG VAL VAL SER VAL LEU THR 95

GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC     1295
VAL LEU HIS GLN ASP TRP LEU ASN GLY LYS GLU TYR LYS CYS LYS VAL 111

TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC     1343
SER ASN LYS ALA LEU PRO ALA PRO ILE GLU LYS THR ILE SER LYS ALA 127

AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG     1391
LYS GLY GLN PRO ARG GLU PRO GLN VAL TYR THR LEU PRO PRO SER ARG 143
```

```
GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC      1439
ASP GLU LEU THR LYS ASN GLN VAL SER LEU THR CYS LEU VAL LYS GLY 159

TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG      1487
PHE TYR PRO SER ASP ILE ALA VAL GLU TRP GLU SER ASN GLY GLN PRO 175

GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC      1535
GLU ASN ASN TYR LYS THR THR PRO PRO VAL LEU ASP SER ASP GLY SER 191

TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG      1583
PHE PHE LEU TYR SER LYS LEU THR VAL ASP LYS SER ARG TRP GLN GLN 207

GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC      1631
GLY ASN VAL PHE SER CYS SER VAL MET HIS GLU ALA LEU HIS ASN HIS 223

TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGATCTAGAG           1677
TYR THR GLN LYS SER LEU SER LEU SER PRO GLY LYS                 235

GGCCCGTTTA AACCCGCTGA TCAGCCTCGA CTGTGCCTTC TAGTTGCCAG CCATCTGTTG    1737
TTTGCCCCTC CCCCGTGCCT TCCTTGACCC TGGAAGGTGC CACTCCCACT GTCCTTTCCT    1797
AATAAAATGA GGAAATTGCA TCGCATTGTC TGAGTAGGTG TCATTCTATT CTGGGGGGTG    1857
GGGTGGGGCA GGACAGCAAG GGGGAGGATT GGGAAGACAA TAGCAGGCAT GCTGGGGATG    1917
CGGTGGGCTC TATGGCTTCT GAGGCGGAAA GAACCAGCTG GGGCTCTAGG GGGTATCCCC    1977
ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTTACGCGC AGCGTGACCG    2037
CTACACTTGC CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA    2097
CGTTCGCCGG CTTTCCCCGT CAAGCTCTAA ATCGGGGCAT CCCTTTAGGG TTCCGATTTA    2157
GTGCTTTACG GCACCTCGAC CCCAAAAAAC TTGATTAGGG TGATGGTTCA CGTAGTGGGC    2217
CATCGCCCTG ATAGACGGTT TTTCGCCCTT TGACGTTGGA GTCCACGTTC TTTAATAGTG    2277
GACTCTTGTT CCAAACTGGA ACAACACTCA ACCCTATCTC GGTCTATTCT TTTGATTTAT    2337
AAGGGATTTT GGGGATTTCG GCCTATTGGT TAAAAAATGA GCTGATTTAA CAAAAATTTA    2397
ACGCGAATTA ATTCTGTGGA ATGTGTGTCA GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC    2457
AGGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCAGG TGTGGAAAGT    2517
CCCCAGGCTC CCCAGCAGGC AGAAGTATGC AAAGCATGCA TCTCAATTAG TCAGCAACCA    2577
TAGTCCCGCC CCTAACTCCG CCCATCCCGC CCCTAACTCC GCCCAGTTCC GCCCATTCTC    2637
CGCCCCATGG CTGACTAATT TTTTTTATTT ATGCAGAGGC CGAGGCCGCC TCTGCCTCTG    2697
AGCTATTCCA GAAGTAGTGA GGAGGCTTTT TTGGAGGCCT AGGCTTTTGC AAAAAGCTCC    2757
CGGGAGCTTG TATATCCATT TTCGGATCTG ATCAGCACGT GTTGACAATT AATCATCGGC    2817
ATAGTATATC GGCATAGTAT AATACGACAA GGTGAGGAAC TAAACCATGG CCAAGTTGAC    2877
CAGTGCCGTT CCGGTGCTCA CCGCGCGCGA CGTCGCCGGA GCGGTCGAGT TCTGGACCGA    2937
CCGGCTCGGG TTCTCCCGGG ACTTCGTGGA GGACGACTTC GCCGGTGTGG TCCGGGACGA    2997
CGTGACCCTG TTCATCAGCG CGGTCCAGGA CCAGGTGGTG CCGGACAACA CCCTGGCCTG    3057
GGTGTGGGTG CGCGGCCTGG ACGAGCTGTA CGCCGAGTGG TCGGAGGTCG TGTCCACGAA    3117
CTTCCGGGAC GCCTCCGGGC CGGCCATGAC CGAGATCGGC GAGCAGCCGT GGGGGCGGGA    3177
GTTCGCCCTG CGCGACCCGG CCGGCAACTG CGTGCACTTC GTGGCCGAGG AGCAGGACTG    3237
```

ACACGTGCTA CGAGATTTCG ATTCCACCGC CGCCTTCTAT GAAAGGTTGG GCTTCGGAAT 3297
CGTTTTCCGG GACGCCGGCT GGATGATCCT CCAGCGCGGG GATCTCATGC TGGAGTTCTT 3357
CGCCCACCCC AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA ATAGCATCAC 3417
AAATTTCACA AATAAAGCAT TTTTTTCACT GCATTCTAGT TGTGGTTTGT CCAAACTCAT 3477
CAATGTATCT TATCATGTCT GTATACCGTC GACCTCTAGC TAGAGCTTGG CGTAATCATG 3537
GTCATAGCTG TTTCCTGTGT GAAATTGTTA TCCGCTCACA ATTCCACACA ACATACGAGC 3597
CGGAAGCATA AAGTGTAAAG CCTGGGGTGC CTAATGAGTG AGCTAACTCA CATTAATTGC 3657
GTTGCGCTCA CTGCCCGCTT TCCAGTCGGG AAACCTGTCG TGCCAGCTGC ATTAATGAAT 3717
CGGCCAACGC GCGGGGAGAG GCGGTTTGCG TATTGGGCGC TCTTCCGCTT CCTCGCTCAC 3777
TGACTCGCTG CGCTCGGTCG TTCGGCTGCG GCGAGCGGTA TCAGCTCACT CAAAGGCGGT 3837
AATACGGTTA TCCACAGAAT CAGGGGATAA CGCAGGAAAG AACATGTGAG CAAAAGGCCA 3897
GCAAAAGGCC AGGAACCGTA AAAAGGCCGC GTTGCTGGCG TTTTTCCATA GGCTCCGCCC 3957
CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC CGACAGGACT 4017
ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG CGCTCTCCTG TTCCGACCCT 4077
GCCGCTTACC GGATACCTGT CCGCCTTTCT CCCTTCGGGA AGCGTGGCGC TTTCTCAATG 4137
CTCACGCTGT AGGTATCTCA GTTCGGTGTA GGTCGTTCGC TCCAAGCTGG GCTGTGTGCA 4197
CGAACCCCCC GTTCAGCCCG ACCGCTGCGC CTTATCCGGT AACTATCGTC TTGAGTCCAA 4257
CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAACAGGA TTAGCAGAGC 4317
GAGGTATGTA GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG CCTAACTACG GCTACACTAG 4377
AAGGACAGTA TTTGGTATCT GCGCTCTGCT GAAGCCAGTT ACCTTCGGAA AAAGAGTTGG 4437
TAGCTCTTGA TCCGGCAAAC AAACCACCGC TGGTAGCGGT GGTTTTTTTG TTTGCAAGCA 4497
GCAGATTACG CGCAGAAAAA AAGGATCTCA AGAAGATCCT TTGATCTTTT CTACGGGGTC 4557
TGACGCTCAG TGGAACGAAA ACTCACGTTA AGGGATTTTG GTCATGAGAT TATCAAAAAG 4617
GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTTT AAATCAATCT AAAGTATATA 4677
TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA TCTCAGCGAT 4737
CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC GTGTAGATAA CTACGATACG 4797
GGAGGGCTTA CCATCTGGCC CCAGTGCTGC AATGATACCG CGAGACCCAC GCTCACCGGC 4857
TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC 4917
AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC 4977
GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG TGTCACGCTC 5037
GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA TCAAGGCGAG TTACATGATC 5097
CCCCATGTTG TGCAAAAAAG CGGTTAGCTC CTTCGGTCCT CCGATCGTTG TCAGAAGTAA 5157
GTTGGCCGCA GTGTTATCAC TCATGGTTAT GGCAGCACTG CATAATTCTC TTACTGTCAT 5217
GCCATCCGTA AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA 5277
GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCGTCAATA CGGGATAATA CCGCGCCACA 5337
TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT TCGGGGCGAA AACTCTCAAG 5397
GATCTTACCG CTGTTGAGAT CCAGTTCGAT GTAACCCACT CGTGCACCCA ACTGATCTTC 5457
AGCATCTTTT ACTTTCACCA GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC 5517
AAAAAAGGGA ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTTCAATA 5577
TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG AATGTATTTA 5637

GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA AAAGTGCCAC CTGACGTC 5695

SEQ ID NO:6

ASP PRO GLU GLU PRO LYS SER CYS ASP LYS THR HIS THR CYS PRO PRO 16
CYS PRO ALA PRO GLU LEU LEU GLY GLY PRO SER VAL PHE LEU PHE PRO 32
PRO LYS PRO LYS ASP THR LEU MET ILE SER ARG THR PRO GLU VAL THR 48
CYS VAL VAL VAL ASP VAL SER HIS GLU ASP PRO GLU VAL LYS PHE ASN 64
TRP TYR VAL ASP GLY VAL GLU VAL HIS ASN ALA LYS THR LYS PRO ARG 80
GLU GLU GLN TYR ASN SER THR TYR ARG VAL VAL SER VAL LEU THR VAL 96
LEU HIS GLN ASP TRP LEU ASN GLY LYS GLU TYR LYS CYS LYS VAL SER 112
ASN LYS ALA LEU PRO ALA PRO ILE GLU LYS THR ILE SER LYS ALA LYS 128
GLY GLN PRO ARG GLU PRO GLN VAL TYR THR LEU PRO PRO SER ARG ASP 144
GLU LEU THR LYS ASN GLN VAL SER LEU THR CYS LEU VAL LYS GLY PHE 160
TYR PRO SER ASP ILE ALA VAL GLU TRP GLU SER ASN GLY GLN PRO GLU 176
ASN ASN TYR LYS THR THR PRO PRO VAL LEU ASP SER ASP GLY SER PHE 192
PHE LEU TYR SER LYS LEU THR VAL ASP LYS SER ARG TRP GLN GLN GLY 208
ASN VAL PHE SER CYS SER VAL MET HIS GLU ALA LEU HIS ASN HIS TYR 224
THR GLN LYS SER LEU SER LEU SER PRO GLY LYS 235

Both FR3exFc and FR1exFc soluble receptors were demonstrated to be expressed to a high level in transiently transfected 293T cells (T-cell antigen infected human embryonic kidney 293 cells). The observation that both soluble receptors remain bound to heparin-coated wells even following extensive washes led the laboratory of the present inventors to try to purify the proteins with the commercial heparin-Sepharose™ resin (Pharmacia). One hundred ml volume supernatants, harvested 48 hours post transfection with either FR3exFc or FR1exFc coding plasmids, were incubated overnight at 4°C with 1 ml heparin-Sepharose™ resin. The resin was washed and then subjected to PBS supplemented with increasing concentration of NaCl. Aliquots of each fraction were analyzed by 7.5% SDS-PAGE stained with GelCode (Pierce) demonstrating a purification profile of more than 90% homogeneity and a peak elution at 400 mM NaCl for FR3exFc (Fig. 3; T=total protein, U=unbound fraction, W=wash). In contrast, FR1exFc was hardly retained on the resin. This result was confirmed by Western analysis of the same fractions with anti-FGFR1ex antibodies demonstrating that most of FR1exFc is in the unbound fraction (not shown).

Functional analysis of FR3exFc and FR1exFc showed that both compete efficiently for FGF9 binding and stimulating FGFR3, thus, demonstrating their potential as inhibitors of FGFRs function and as a target (FR3exFc) for selecting FGFR3 inhibitory molecules.

Neutralizing effect of soluble receptors

The ability of hFR3-TDhis and FR3exFc to inhibit FGF-dependent FDCP-R3 cell proliferation was compared. Both soluble receptors inhibited FDCP-R3 cell proliferation, however, FR3exFc was about 60 times more potent than hFR3TDhis (Fig. 4; legend: ♦-FDCP-FR3$^{23-374}$TDhis on FDCP-FR3 cells + FGF9, ■-FR3exFc on FDCP-FR3 cells + FGF9, ▲- FDCP-FR3$^{23-374}$TDhis on FDCP-FR3 cells + IL, X- FR3exFc on FDCP-FR3 cells + IL3). Neither had an effect on FDCP cells stimulated with IL3. The fact that FR3exFc is entirely in dimeric form whereas only a small proportion (1/10) of hFR3$^{23-374}$TDhis is in a dimeric form might explain, at least in part, this difference.

Example 3: Screening for Antibodies

Panning and first screening of Ab Binding Characterization

The screening strategies to identify Fabs from the Human Combinatorial Antibody Library (HuCAL®), developed at MorphoSys, Munich, Germany and disclosed in WO 97/08320, US patent 6,300,064, and Knappik et al., (2000), the entire contents of which are incorporated herein by reference, using soluble dimeric forms of the extracellular domain of the FGFR3 receptor are shown in Table 2.

TABLE 2

Panning Strategies

	Panning Round 1	**Panning Round 2**	**Panning Round 3**
Screen 1	**FR3-TDhis**	**HEK293**	**FR3-TDhis**
Screen 2	**FR3exFc** captured with mouse anti-human IgG	**RCJ-FR3ach**	**FR3exFc** captured with mouse anti-human IgG
Screen 3	**FR3-TDhis** (Round 1 of panning 1)	**RCJ-FR3ach & RCJ-FR3wt**	**FR3exFc** Captured with mouse anti-human IgG

The screening was carried out, for example in Screen 1, by coating the wells of a 96 well plate with hFR3$^{23-374}$TDhis (FR3-TDhis), panning with the bacteriophage library and

selecting the positive clones. The positive clones were then tested on HEK293 (293, human embryonic kidney) cells, expressing endogenous FGFR3. The positive clones were selected and rescreened on FR3-TDhis. Two additional similar screenings were carried out as shown in Table 2. In screen 2 the first and third pannings were carried out with the FR3exFc antigen and the second panning carried out with RCJ cells expressing a mutant (achondroplasia) form of FGFR3.

An overview of the number of initial hits and of the candidate clones is shown in Table 3.

Table 3

Overview of Screenings 1, 2 and 3 on FGFR3

	screened clones	primary hits	sequenced clones	consolidated candidate clones (ELISA & FACS)
Screen 1	1076	208	69	15 MSPRO 1-15
Screen 2	864	300	32	22 MSPRO 20-33 and 52-59
Screen 3	768	487	52	11 MSPRO 40-50

<u>Sequence and Vector Data</u>

A plasmid map and sequence (SEQ ID NO:52) of the dHLX-MH vector are presented in Fig. 28A and 28B.

Figure 29A shows the plasmid map of the phage display vector used in accordance with the present invention. Figure 29B is the corresponding sequence and restriction digest map (SEQ ID NO:53).

Figure 30 displays the polynucleotide sequences of the specific VL and VH domains of MSPRO2 (SEQ ID NO:74 and 84); MSPRO11 (SEQ ID NO:70 and 85), MSPRO12 (SEQ ID NO:75 and 89); MSPRO21 (SEQ ID NO:67 and 78); MSPRO24 (SEQ ID NO:64 AND 79); MSPRO26 (SEQ ID NO:71 AND 86); MSPRO28 (SEQ ID NO:62 AND 80); MSPRO29 (SEQ ID NO:65 AND 87); MSPRO54 (SEQ ID NO:73 AND 82); MSPRO55 (SEQ ID NO:69 AND 83); MSPRO59 (SEQ ID NO:76 AND 91). The sequences include the framework domains 1-4 and the CDR domains 1-3. SEQ ID NO:61, 63, 66, 68, and 73

denote the polynucleotide sequences of the parent VL (kappa or lambda) strands. SEQ ID NO:77, 81, 88 and 90 denote the polynucleotide sequences of the VH parent strands.

Example 4:Analysis of Fabs identified by first screening.

Specificity of Antibody recognition

The first screening yielded 15 different Fabs that specifically recognize FGFR3 *in vitro* and on the cell surface. Fourteen of these were produced and sent to ProChon for further analysis. LY6.3, an anti-lysosyme antibody, was isolated from the same library and serves as a control. ELISA analysis, according to the following protocol was carried out to determine the specificity of the isolated Fabs for FGFR3 or FGFR1.

Fab-FR3/Fc Binding Assay

MaxiSorp ELISA plates were coated with 100 µl anti-human Fc (10 µg/ml) in bicarbonate overnight at 4°C. Wells were washed five consecutive times with a PBS solution containing 0.1% Tween 20 (PBST). The well surface was blocked with 250 µl PBST+3%BSA (blocking solution) for 1 hour at 37°C. This was followed by capturing 1 µg of FGFR/Fc for 1 hour at room temperature. To assess the antibody binding to the captured FGFR/Fc, 1 µg each of the tested Fabs was incubated in 100 µl blocking solution per well 1 hour at room temperature Wells were washed 5 times with PBST. Reaction was initiated with the addition of 100 µl of 0.8µg/ml goat anti-human Fab-HRP diluted in blocking solution, subsequently washed and detected with TMB substrate (Pierce). The absorbance was measured at 450 nm. A comparison of ELISA analyses done in the laboratory of the present inventors (Prochon) and at MorphoSys is presented in the following Table 4.

TABLE 4

ProChon	MorphoSys			
	FR1/Fc	FR3/Fc	FR1/Fc	FR3/Fc
MS-PRO1	++	++	+/-	+
MS-PRO2	-	++	-	++
MS-PRO3	+	++	-	++
MS-PRO4	-	+	-	++
MS-PRO5	-	++	+/-	+
MS-PRO6	-	++	-	+
MS-PRO7	-	++	-	+
MS-PRO8	+	++	-	+
MS-PRO9	-	+/-	+/-	+
MS-PRO10	+	++	-	++
MS-PRO11	-	+/-	+	++
MS-PRO12	-	+/-	-	++
MS-PRO13	-	+/-	+/-	+
MS-PRO14	-	-	-	+
LY6.3 (control)	-	-		

In most cases, the data generated at Morphosys and in the laboratory of the present inventors are in agreement. However, some Fabs behave differently. For example, MS-PRO3 and 10 were found to be completely FGFR3 specific under Morphosys conditions. In the laboratory of the present inventors, both show considerable cross-reaction with FGFR1. The FACS analysis, done at Morphosys, supports the Prochon results for MS-PRO3 but not for MS-PRO10. Taking into account the potency and specificity of the Fabs, MS-PRO2 has the highest score according to these preliminary data.

Example 5: Affinity of Fab to FGFR3

The affinity measurements were performed by BIAcore according to the standard procedure recommended by the supplier (Pharmacia). The anti-Fc antibody was coupled via the EDC/NHS chemistry to the chip and subsequently FGFR3 was captured. The Fabs of the invention were then bound to this surface.

Table 5 shows a comparison of affinities of Fabs candidates to FGFR3 as determined by BIAcore and by FACS-scatchard.

Table 5

Comparison of Antibody Affinities to FGFR3 determined by BIAcore and FACS-Scatchard

Fab clone	BIAcore [nM]	Indirect FACS-Scatchard [nM]
MSPRO2	37 ± 10	43
MSPRO11	4 ± 2	4
MSPRO12	14 ± 2	6.5
MSPRO21	9 ± 2	0.6
MSPRO24	10 ± 2	0.3
MSPRO26	4 ± 1	1.4
MSPRO28	9 ± 0.4	0.3
MSPRO29	6 ± 4	0.4

Table 1E shows the affinity as determined by BIAcore for the Fab candidates shown in Table 5 converted into the Fab mini-antibody format, Fab-dHLX-MH, where a dimer of the Fab monomer is produced after insertion into an expression vector as a fusion protein.

Table 6 shows the results of a competition assay wherein each MSPRO Fab was bound to the FGFR3 at a concentration of 500nM or 1, 000 nM and coinjected in pairs with the other MSPRO Fabs. The (-) indicates binding to the same or nearby epitope while (+) indicates binding to different epitope. The results show that MSPRO2 and12 bind to the same or nearby epitope while MSPRO11, 21, 24, 26, 28 and 29 bind to an epitope different from that of MSPRO2 or 12.

__Example 6: Specific Neutralizing Activity of the Antibodies__

A: __FDCP Cell Proliferation Assay__

The FDCP cell line is a murine immortalized, interleukin 3 (IL3) dependent cell line of myelocytic bone marrow origin, which does not express endogenous FGF Receptors (FGFR).

Upon transfection with FGFR cDNA, the FDCP cell line exhibits an FGF dose dependent proliferative response that can replace the dependence on IL3. FDCP cell lines, transfected with FGFR cDNAs can therefore be used to screen for specific inhibitors or activators of FGFR, as well as for analyzing FGFR signaling. The FDCP cell response to various ligands was quantitated by a cell proliferation assay with XTT reagent (Cell Proliferation Kit, Biological Industries Co.). The method is based on the capability of mitochondrial enzymes to reduce tetrazolium salts into soluble colored formazan compounds which can be quanititated and is indicative of cell viability. Specifically, FDCP cells expressing FGFR3IIIb, FGFR3IIIc or FGFR1 were grown in "full medium" (Iscove's Medium containing 2ml glutamine, 10% FCS, 100ug/ml penicillin, 100ug/ml streptomycin) supplemented with 5ug/ml heparin and 10ng/ml FGF9. Cells were split every 3 days and kept in culture no more than one month. One day prior to the experiment, the cells were split. Before the experiment, the cells were washed 3 times (1000 rpm, 6 min) with full medium. Later, the cells were resuspended and counted with Trypan Blue. Twenty thousand (20,000) cells /well were added to wells in a 96-well plate in 50ul in full medium containing 5 ug/ml heparin. Conditioned medium was added in an additional volume of 50ul full medium containing FGF9 at varying concentrations to a final volume of 100ul. A primary stock solution (usually 2x the higher concentration) of the antibody (or Fabs) was prepared in Iscove's+++ containing 5μg/ml heparin and 2.5ng/ml FGF9 or IL-3 (final concentration 1.25 ng/ml). Dilutions were filtered in a 0.2 μm syringe nitrocellulose filter blocked first with 1mg/ml BSA and washed then with Iscove's+++. Aliquots of requested serial dilutions were prepared. Dilutions were kept on ice until use. 50 μl of the corresponding 2x final concentration was added to each well and the plate was incubated at 37^0C for either 40 hours or either 64 hours. After incubation, the reaction was developed as follows: 100 μl of activator solution was added to 5 ml XTT reagent and mixed gently. 50 μl of mixture was added to each well. Optical density (OD) at 490 nm at this point gave the zero time reading. Cells were then incubated at 37^0C for 4 hours (in the case of a 40 hour incubation) or 2 hours (in the case of a 64 hour incubation) and proliferation was measured by O.D. at 490 nm (A490).

It is noted that the assay is successful when the O.D. of untreated control growing with saturated amounts of FGF (10 and 20 ng/ml) is at least 1.3 O.D. units. Furthermore, it is noted that the background of wells with no cells should be 0.2-0.35 O.D. units and that the

O.D. absorbance of 1.25 ng/ml FGF9 should not be less than 40% of the O.D. absorbance achieved with saturated FGF 9 concentration (10 and 20 ng/ml). Specific inhibition of FGF and FGF receptor mediated proliferation should always be accompanied with lack of any inhibition of the same antibody concentration on IL-3 dependent cell proliferation.

The following FDCP cell lines were used:

*FDCP-C10: FDCP cells transfected with the human wild-type FGF receptor 3IIIc.

*FDCP-R3: FDCP cells transfected with the human wild-type FGF receptor 3IIIb.

*FDCP-R1: FDCP cells transfected with the human wild-type FGFR1.

*FDCP-F3Ach: FDCP cells infected with human FGFR3 mutated at amino acid Glycine 380 to Arginine (G380R), analogous to the most common human achondroplasia mutation.

<u>B: Neutralizing activity</u>

The neutralizing activity of the antibodies was measured by the aforementioned cell proliferation analysis in FDCP-FR3 and FDCP-FR1 cell lines. Increasing amounts of the indicated Fabs (MSPRO 2, 3 and 4) were added to FDCP-FR3 (closed triangle ▲(2), star * (3), and circle ● (4)) or FDCP-FR1 (diamond ♦ (2), square ■ (3) and open triangle Δ(4)) grown in the presence of FGF9 (Fig. 5). Two days later, an XTT proliferation assay was performed. While none of the Fabs inhibited FDCP-FR1 cell proliferation, MSPRO2 and 3 inhibited FDCP-FR3 proliferation with a similar IC50 of about 1.0 μg/ml (Fig. 5). In contrast, MS-PRO4 had no inhibitory effect on FDCP-FR3 proliferation. These data are in agreement with those generated at Morphosys. The rest of the Fabs were similarly analyzed on FDCP-FR3 expressing cells. Increasing amounts of the indicated Fabs were added to FDCP-FR3 grown in the presence of FGF9 (Fig. 6). The results of the proliferation assay done at Morphosys and at Prochon are compared in Table 6. (NA- data not available)

Table 6

	Prochon		Morphosys	
	FDCP-FR1	FDCP-FR3	FDCP-FR1	FDCP-FR3
MSPRO1	-	++	NA	NA
MSPRO2	-	++	NA	++
MSPRO3	-	++	NA	++
MSPRO4	-	-	NA	-
MSPRO5	-	+	NA	+
MSPRO6	-	-	NA	+/-
MSPRO7	-	++	NA	+
MSPRO8	-	+/-	NA	+/-
MSPRO9	-	+	NA	+
MSPRO10	-	+	NA	NA
MSPRO11	-	+++	NA	++
MSPRO12	-	+++	NA	+++
MSPRO13	-	-	NA	NA
MSPRO14	-	-	NA	NA
LY6.3	-	-	NA	NA

As shown in Table 6, there is an excellent agreement between the Prochon and Morphosys data. About half of the Fabs show considerable neutralizing activity, MSPRO12 being the most potent. Most of the inhibitory Fabs performed well in the binding assay (Table 4), with MSPRO11 and MSPRO12 being the exception to the rule, however, clearly remain good candidates to pursue. None of the Fabs (including those that crossreact with FGFR1) inhibited FGF-dependent FDCP-FR1 proliferation. In addition, FDCP-FR3 grown in the presence of IL3 were not affected by any of the Fabs.

An additional 20 new Fabs were selected from the second panning done at Morphosys. Three of these new Fabs (MSPRO52, MSPRO54 and MSPRO55) were subjected to the FDCP cell proliferation test and all were found to neutralize the receptor (Fig. 7A). Interestingly (and in

accord with MorphoSys affinity data), one Fab (MSPRO54) showed strong neutralizing activity against FGFR1 (Fig. 7B).

Example 7: Receptor Expression and Activation in RCJ Cells

RCJ cell assay

RCJ cells (fetal rat calvaria-derived mesenchymal cells, RCJ 3.1C5.18; Grigoriadis, 1988) were generated to express various FGF Receptors an inducible manner, in the absence of tetracycline. The RCJ-M14 line (RCJ-FR3ach) expresses FGFR3-ach380 mutant upon induction by the removal of tetracycline. The cells were incubated in low serum after which FGF was added to stimulate receptor function and signaling. The cells were lysed and the receptor level, receptor activation and signaling are assessed by Western with anti-FGFR3 (Santa Cruz), anti-phospho-tyrosine (Promega), and anti-active ERK (or JNK) (Promega) respectively.

RCJ-M14 cells were grown in α-MEM supplemented with 15% fetal calf serum, 1x penicillin/streptomycin/nystatin, 1x glutamine, 600 μg/ml neomycin, 2 μg/ml tetracycline, 50 μg/ml hygromycin B to subconfluence. The medium was aspirated off and the cells washed with trypsin, 1 ml/10 cm dish, then trypsinized with 0.5 ml/10 cm dish. The cells were resuspended in 10 ml α-MEM supplemented with 15% fetal calf serum, 1x penicillin/streptomycin/nystatin, 1x glutamine, 600 μg/ml neomycin, and 2 μg/ml tetracycline.

Sixty thousand ($6x10^5$) cells/well were seeded in a 6-well dish. Alternatively, twice that number may be seeded. The cells were washed thrice 24 hours later (or 8 hours later if twice the amount of cells are seeded) with 1 ml α-MEM, and then incubated with α-MEM supplemented with 15% fetal calf serum, 1x penicillin/streptomycin/nystatin, and 1x glutamine (induction medium) for 16 hours. Cells were washed thrice with 1 ml α-MEM and allowed to grow for 4 additional hours in 1 ml of 0.5% exhausted serum (prepared by diluting the induction medium X30 with α-MEM).

FGF9 (1 ng/ml) was added for 5 minutes and cells are then placed on ice. The cells were washed twice with ice-cold PBS and then lysed with 0.5 ml lysis buffer. The cells are scraped into an eppendorf tube, vortexed once and placed on ice for 10 minutes. The lysate was microcentrifuged 10 minutes at 4^0C and the cleared lysate transferred into a fresh Eppendorf tube.

The protein content was determined by Bradford or DC protein assay (Bio-Rad, cat# 500-0116 - see manufacture instructions). Total protein aliquots, supplemented with 1/5 volume of 5x sample buffer, were boiled for 5 minutes and stored at -20^{0}C until ready to load on gel. In parallel an immunoprecipitation (IP) assay was performed, 10 μl anti- FGFR3 antibodies were added to the rest of the lysates and incubated for 4 hours at 4^{0}C. 40 μl protein A-Sepharose was added and incubated for 1 hour at 4^{0}C with continuous shaking. Afterwards, the mixture was microcentrifuged 15 seconds, and the fluid was aspirated, carefully leaving a volume of ~30 μl above the beads. The beads were washed 3 times with 1 ml lysis buffer. At this step, the protease inhibitor mix is omitted from the buffer.

After the final wash, 15 μl of 5x sample buffer was added, samples were boiled 5 minutes and stored at -20^{0}C until ready to load onto gel. Samples were loaded on 7.5% SDS-PAGE, cast on a Mini-PROTEAN II electrophoresis cell, and run at 100 V through the upper gel and at 150 V through the lower gel. Proteins were transferred onto nitrocellulose sheet using the Mini trans-blot electrophoretic transfer cell at 100 V for 75 minutes or at 15 V overnight. The lower part of the total lysate Western blots was probed with anti-active MAPK (ERK) and the upper part is probed with anti-phosphotyrosine, both diluted 5x10^3. The IP lysate Western blots were probed with anti-anti-phosphotyrosine (R&D Systems). Hybridization was detected by ECL following the manufacturer's instructions.

BIAcore and proliferation analyses done at MorphoSys showed that among the new Fabs, MS-PRO54 is highly cross reactive with FGFR1. To further test the cross reactivity of the new Fabs, RCJ cells expressing either FGFR3ach (RCJ-M14; M14 on figure 9A) FGFR3 wild type (W11 on figure 9B), FGFR1 (R1-1 on figure 9C) or FGFR2 (R2-2 on figure 9D) were incubated with increasing amount of MS-PRO54 and MS-PRO59 for 1 hour. FGF9 was added for 5 minutes and cell lysates were analyzed by Western for pERK activation (Figs. 8A-B, 9A-9D). Figure 8A shows that MSPRO2 and MSPRO12 block FGFR3 receptor activation in W11 and RCJ-FR3ach expressing cells. Furthermore MSPRO13 was able to block FGFR1 activation while none of the Fabs blocked FGFR2 activation. Figures 8B and 9A-9D show the results of several Fabs on RCJ expressing wildtype FGFR3 (8B) or the different FGFR types. MSPRO29 appeared as the best FGFR3 blocker and was also effective in blocking FGFR1 (Fig. 9c); however, MSPRO54 was the most effective Fab against FGFR1. None of the Fabs significantly inhibited FGFR2 activity. There are only a few amino acid residues, within the third Ig domain, that are shared by FGFR3 and FR1 but not by FR2.

Making mutants at these sites should clarify their role in Fab-receptor binding. Figure 8B depicts the dose effect of MS-PRO12, 29 and 13, stimulated with FGF9 and analyzed by Western blot using anti-ERK antibodies. . MSPRO29 strongly inhibits FGFR3 activation (5ug), MSPRO12 has an inhibitory effect but at a higher concentration (50 ug).

Example 9: Epitope mapping of selected Fabs

Constructs containing cDNAs that code for segments of the extracellular domain of FGFR3 were generated (Fig. 10). D2 comprises Ig domain 2, D2-3 comprises Ig domains 2 and 3 and D1-3 comprises Ig domains 1, 2 and 3.These include pChFR3^{D2}Fc that codes for Ig-like domain 2 of FGFR3 and pChFR3^{D2,3}Fc that codes for domain 2 and 3, both as human Fc fusions. The corresponding chimeric proteins, as well as the control hFR3exFc (containing domains 1, 2 and 3) were anchored to an ELISA plate coated with α human Fc antibody. A panel of 8 best Fabs, MSPRO2, 11, 12, 21, 24, 26, 28 and 29, were added, and bound Fab was determined with HRP-α human Fab (Fig. 11). The results in Fig. 11 demonstrate that MSPRO2 (speckled bar) and MSPRO12 (hatched bar) differ from the other tested Fabs. Both bind to the Ig like domain 2 while the others require domain 3 for binding. It was then tested whether or not Fabs that belong to the second group would distinguish the FGFR3IIIc isoform from the FGFRIIIb from. FDCP-FR3IIIb or FDCP-FR3IIIc cells were incubated in the presence of 1.25 ng/ml FGF9 with increasing doses of either MSPRO12 or MSPRO29. Ly6.3 was included as control. After 2 days in culture, cell proliferation was measured with the XTT reagent. Clearly, MSPRO29 (open triangle) was completely ineffective against the IIIb isoform (Fig. 12). In contrast, MSPRO12 (square on hatched or solid lines) was equally effective against both isoforms. These data suggest that residues that differ between the two isoform are critical for MSPRO29 (and probably also for the other Fabs in the same group) FGFR3 binding.

Domains in FGFR3 recognized by the new Fabs.

In agreement with data generated at Morphosys, MSPROs can be divided into 2 groups, one that includes Fabs that bind the FGFR3 Ig II domain (MSPRO2 and 12) and a second with members that require the Ig III domain for binding (MSPRO11, 21, 24, 26, 28, and 29). To classify the new Fabs obtained from the last screen performed at Morphosys, as well as some previously obtained Fabs, a proliferation assay of FDCP cells expressing either FR3IIIb or FR3IIIc was performed. The cells were incubated in the presence of 10 (IIIb) or 5 (IIIc)

ng/ml FGF9 with increasing doses of the indicated Fabs. After 2 days in culture, cell proliferation was measured with the XTT reagent.

In agreement with Morphosys data, MSPRO59 efficiently inhibited both FDCP-FR3IIIb (Fig. 13A) and FDCP-FR3IIIc cells (Fig. 13B) while MSPRO21, 24, 26, 28, 29 and 54 inhibited FDCP-FR3IIIc proliferation only.

Example 10: Bone culture

Radiolabeled MSPRO29 was used to determine whether or not MSPRO Fabs can enter the bone growth plate.

To determine the effect of iodination on Fab activity, 50 μg of MSPRO29 was first labeled with cold iodine using Pierce IodoGen coated tubes. The process was carried out either without iodine, with 0.04 mM or with 1 mM NaI. MSPRO29 was then purified through a sephadex G-50 column. The ability of the modified Fab to bind FGFR3 was determined by ELISA. MaxiSorp wells were coated with anti-human Fc. FGFR3/Fc (checkered bars) was then anchored to the wells. In parallel, a similar set of wells was left in blocking buffer only (no FR3/Fc, hatched bars). The unmodified (no I) or the modified MSPRO29 (low for that labeled with 0.04 mM NaI (low) and high for that labeled at 1 mM NaI (high); 2 G-50 fractions each) were added at approximately 5 μg/well and binding was measured with anti-human Fab. Fresh MSPRO29 and buffer alone were included as controls (Fig. 14)..

MSPRO29 labeled in the presence of 0.04 mM NaI showed equal binding to the receptor as compared to the control unmodified Fab MSPRO29 labeled in the presence of 1 mM NaI (high I) also bound the receptor, however, the noise level of this sample was as high as the signal itself suggesting that at the high Iodide concentration the Fab was inactivated.

The neutralizing activity of the modified Fab was tested in a proliferation assay using FDCP-FR3 (C10) (Fig. 15). FDCP-FR3 (C10) cells were treated with the indicated amount of labeled or unlabeled (without I) MSPRO29. The proliferation rate of the cells was determined by XTT analysis. The Fab was labeled at either 0.04 mM (Low) or 1 mM NaI (High). Two G-50 fraction (I and II) were analyzed. Fresh MSPRO29 and buffer alone (mock) were included as controls.

This experiment showed that MSPRO29, labeled at 0.04 mM NaI, kept its activity almost entirely while that labeled at 1 mM NaI lost its activity completely. MS-PRO29 was labeled with 1 mCi ^{125}I. The specific activity of the Fab was 17 μCi/μg.

Ex vivo distribution of ^{125}I MSPRO29 in bone culture

Femora prepared from newborn mice were incubated with 2 μg ^{125}I-MSPRO29 (17 μCi/μg) or ^{125}I-Ly6.3 (20 μCi/μg) for 1, 3 or 5 days in culture. Then, sections were processed for radiomicroscopy. After 3 days in culture, MSPRO29 was predominantly visualized at the higher hypertrophic zone and to a lesser extent at the secondary ossification region (Figs. 16A-16F). Hematoxylin-eosin staining of growth plate treated with radiolabelled MS-PRO29 or Ly6.3 (Figs. 16A and 16D, respectively) x100 magnification. Radiomicoscopic sections of growth plate treated with radiolabelled MS-PRO29 or Ly6.3 (Figs. 16B and 16E) at X100 magnification. Figs. 16C and 16F are the same as Figs. 16B and 16E but at x400 magnification. The arrow in figure 16C indicates the location of the specific binding of the radiolabelled MS-PRO29 to the higher hypertrophic zone of the growth plate.

As compared to MSPRO29, the control Ly6.3 Fab was weakly and evenly distributed throughout the whole growth plate. At day 1 in culture, the signal was weaker but with similar distribution pattern. This distribution also holds at 5 days in culture with a less favorable signal to noise ratio (data not shown). This clearly demonstrates that MSPRO29 binds FGFR3 in our target organ.

Example 11: Neutralizing Activity on Constitutively Activating Receptors

The inhibitory activity of MSPRO antibodies on ligand-dependent and ligand-independent FDCP proliferation expressing FGFR3 Achondroplasia mutation was tested.

A proliferation assay was carried out using FDCP-FR3wt (C10) or FDCP-FR3ach cells incubated with 1.25 or 5 ng/ml FGF9 respectively and with increasing amounts of MSPRO54 or MSPRO59. As shown in Fig. 17, both MSPRO54 (diamond) and 59 (square) antibodies neutralize the mutant receptor. Few of the FDCP-FR3ach acquired ligand independent cell proliferation due to the high expression of the FGFR3ach mutation.

FDCP cells that express the achondroplasia FGFR3 (FDCP-FR3ach) and proliferate independently of ligand were incubated with the indicated amount of MSPRO12, 29, 59 or the control Ly6.3. Two days later, cell proliferation was determined by an XTT analysis. When inhibition of cell proliferation by the MS-PRO 12, 29, 54 and 59 were tested, only the antibodies 12 and 59 (the only Ab which recognized D2 domain) inhibited the ligand-independent cell proliferation (Figs. 18A and 18B). Previously, the activity of MSPRO Fabs generated in the first and second screens (MSPRO1-15 and MSPRO21-31, respectively) by XTT analysis of FDCP-FR3ach cells were tested. These cells, when generated, show ligand-

dependent proliferation. With time, however, they acquired a ligand-independent ability to proliferate. Accordingly, neutralizing Fabs were able to block the ligand-dependent, but not the ligand-independent, proliferation of these cells. To show whether this is also true for the new batch of Fabs, FDCP-FR3ach cells, which is the FDCP-dervied cell line that expresses a constitutive FGFR3-G380R (Ach), were subjected to XTT analysis in the presence of MSPRO59 and MSPRO29. Surprisingly, and in contrast to the ineffective MSPRO29 (triangle), MSPRO59 (diamond) completely blocked cell proliferation (Fig. 18B). Whether other Fabs that, like MSPRO59, bind to the second Ig like domain would also inhibit FDCP-FR3ach cell proliferation was tested next. Indeed, it was found that MSPRO12 strongly inhibits the constitutive cell proliferation. However, the third member in this family, MSPRO2, had no effect on either the constitutive or the ligand-dependent cell growth, suggesting that the Fab may have lost its neutralizing activity (not shown).

Example 12: RCS Chondrocyte Culture

Effect of Fabs on growth arrest of RCS Chondrocytes

RCS is a rat chondrosarcoma derived cell line expressing preferentially high levels of FGFR2 and FGFR3 and low levels of FGFR1 (Sahni, 1999). In this cell line FGFR functions as an inhibitor of cell proliferation similar to its expected role in the achondroplasia phenotype. Analysis of RCS cell proliferation mediated by the addition of different molecules of the invention, showed that MSPRO54 and MSPRO59 were able to restore cell proliferation. The screening was performed on RCS parental cells in 96 wells plates. Cells were seeded at a concentration of 2,000 cells/well. The following day 10ng/ml FGF-9 and 5μg/ml heparin were added to the cells. 50ug/ml of the antibodies were added. Positive and negative controls for cell proliferation are included in this assay at the same concentrations as the tested molecules. On the fourth day of incubation, plates were observed under the microscope. If all cells were viable, no quantitative assay to measure the effect of the variants was performed. If cell death was observed, the Cy-Quant assay kit is used to measure the amount of the cells. The results are measured in a fluoro ELISA reader. Figure 19 shows the ELISA results in bar graph form. Untraeted cells are shown speckled, ligand treated cells are shown in gray, control antibody (LY6.3)treated cells are in blak while MSPRO54 and MSPRO59 treated cells are shown in hatched otr checkered bars, respectively.

Example 13: *Ex vivo* Bone Culture

The femoral bone cultures were performed by excising the hind limbs of 369-mice, heterozygous or homozygous mice for the achondroplasia G369C mutation (age P0). The limbs were carefully cleaned up from the surrounding tissue (skin and muscles) and the femora exposed. The femora were removed and further cleared from tissue remains and ligaments. The femora were measured for their initial length, using a binocular with an eyepiece micrometer ruler. The bones were grown in 1 ml of medium in a 24 well tissue culture dish. The growing medium is α-MEM supplemented with penicillin (100 units/ml), streptomycin (0.1 mg/ml) and nystatin (12.5 units/ml). In addition, the medium contains BSA (0.2%), α-glycerophosphate (1 mM) and freshly prepared ascorbic acid (50 μg/ml). The bones were cultured for 15 days. Measurements of bone length and medium replacement were performed every three days.

At the end of the experiment, the growth rate of the bones was determined. The growth rate of bones is calculated from the slope of a linear regression fit on the length measurements obtained from day 3 to 9.

The results shown in Fig. 20 demonstrate a dose dependent increase in the growth rate of bones treated with MS-PRO 59 in comparison to non-relevant control LY6.3 Fab. The LY6.3-treated control femurs, marked with a circle, grew at the slowest rate. The MSPRO59 treated femurs exhibited a higher growth rate, with the optimal rate achieved at MSPRO59 concentration of 100ug/ml (square) while the higher cocentration (400ug/ml, triangle) showed inhibition. Moreover, the growth rates achieved by 400 microgram/ml of MSPRO59 doubled in comparison to the control Ab (3.55 U/day as compared to 1.88 U/day, respectively). This experiment shows the neutralizing effect of the MSPRO59 antibody on constitutively active FGFR3, in an *ex vivo* model.

Example 14: *In-vivo* trials

FDCP-FR3ach cells, but not FDCP (control) cells, were found to be tumorigenic when injected into nude mice. Each of 9 mice received two sub-cutaneous injections with different amount of transfected cells. Fourteen days after injection, progressively growing tumors started to appear at the site of FDCP-FR3ach injection but not at the FDCP site of injection. External examination of the tumors showed a high vascular capsule. ^{125}I-labeled MSPRO59 and LY6.3 were injected I.P. into nude mice carrying the FDCP-FR3ach derived tumor. The

tumors were dissected 4 and 24 hrs later and radioactivity was measured. Concentration of MSPRO59 Abs in FDCP-FR3ach derived tumors is shown in Fig. 22.

Example 15: Animal Model for Bladder Carcinoma

Recent studies have shown that the IIIb isoform of FGFR3 is the only form expressed in bladder carcinoma, in particular an FGFR3 with an amino acid substitution wherein Serine 249 is replaced by Cysteine (S249C). The progression of the cancer is believed to be a result of the constitutive activation resulting from this amino acid substitution. In order to create the FGFR3 IIIb form, we isolated the IIIb region of FGFR3 from HeLa cells and generated a full length FGFR3IIIb isoform in pLXSN. Retroviruses, expressing either normal FGFR3 (FR3wt) or mutant FGFR3 (FR3-S249C) were produced and used to infect FDCP cells. Stable pools were generated and further used for *in-vitro* and *in-vivo* experiments.

A. MSPRO59 reduces tumor size in mice

Twelve nude mice were injected with $2x10^6$ FDCP-S249C cells subcutaneous at 2 locations, one on each flank. A week later MSPRO59 was administered i.p. at 400ug per mouse (3 mice in total), followed by 3 injections of 275 ug each, in 2 to 3 days intervals. Following 24 and 26 days the tumor size was measures. Figure 23 shows the inhibitory effect of MSPRO59 on tumor size.

B. Treating FDCP-S249C-derived tumors with MSPRO59

Nude mice (3 in each group), were injected subcutaneous at 2 locations, one on each flank, with $2x10^6$ FDCP-S249C cells each. A week later, 400 or 80 μg MSPRO59 were injected IP. Three days later, mice were injected with 400 μg followed by 5 additional injections with 275 μg MSPRO59, each, every 3 or 4 days. Mice initially treated with 80 μg MSPRO59 were similarly given an additional 80 μg MSPRO59 followed by 5 injections with 50 μg MSPRO59 at the same schedule. Mice injected with PBS were used as control. Tumors typically appeared three weeks post injection of the cells. Tumor volume was estimated from measurements in 3 dimensions at 16,20, 23 or 32 days post cell injection.

As shown in Figure 24 there is both a delay in tumor appearance and an inhibitory effect on tumor progression in the treated mice. This indicates that these FGFR3 inhibitors are potent *in-vivo*.

These data may also help us understand the mechanism by which the S249C-derived tumors were developed. Since we are using pools of cells, treatment with MSPRO59 inhibited the

susceptible cells, leading to delay in tumor appearance. However, over time, the resistant cells survived and proliferated, giving rise to a solid tumor.

C. MSPRO59 inhibits FDCP-FR3ach380 derived tumor growth.

Nude mice were injected subcutaneously in the flank with $2x10^6$ FDCP-FR3ach380 cells, each. Treatment with MSPRO59 began at the day of tumor appearance. Three mice were treated with a known tyrosine kinase inhibitor (TKI -50 mg/Kg/injection) and three with 400 μg followed by 3 additional injections with 300 μg MSPRO59, every 3 or 4 days. Three mice were treated with PBS alone as control. The tumor size was estimated as before at the indicated days after cell injection. The dose schedule is shown in Table 7 below.

Table 7

	Days After FDCP-FR3^{ach380} Cell Injection			
	21	25	28	31
MSPRO59 (μg)	400μg	300μg	300μg	300μg
PBS (μl)	50	50	50	50

Results are shown in bar graph format in Figure 25A.

D. MSPRO59 inhibits FDCP-S249C induced tumor growth

To overcome the instability of the FDCP-derived pools, clones from each pool FDCP-S249C clone #2) were isolated and characterized. These clones were tested in an XTT proliferation assay and were shown to be inhibited by MSPRO59. $2x10^6$ cells from each clone were injected into nude mice. Tumors appeared 18-30 after injection.

FDCP-S249C clone #2 was injected subcutaneously on the flank. A week later mice were injected with 280 μg MSPRO59 single chain (SC) I.P. every day. Mice injected with PBS were used as control. Tumor volume was estimated from measurements in 3 dimensions at 18 or 24 days post cell injection. An apparent inhibition of tumor growth by MSPRO59(SC) was observed in tumors derived from clone 2 (figure 26). Figure 25B shows the inhibition effected by MSPRO59scFv and MSPRO59 Fab compared to the control. Both inhibit growth of the tumor resulting from constitutively activated cells.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by references.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

REFERENCES

Ansel et al, Pharmaceutical Dosage Forms and Drug Delivery Systems, 5th Ed. (Lea & Febiger 1990)

Ausubel et al (Eds), Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (New York) (1987-1999)

Bellus et al., Nature Genetics, 14:174-176 (1996)

Better et al, ", Science 240d(4855):1041-1043 (1988)

Blume-Jensen et al., Nature 411:355-365 (2001)

Boulianne et al, Nature 312(5995):643-646 (1984)

Burchiel et al., Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, Burchiel and Rhodes, eds., Masson Publishing Inc. (1982).

Cappellen et al., Nature Genetics, 23:18-20 (1999)

Chesi et al., Blood, 97(3):729-736 (2001)

Colligan et al (eds.), Current Protocols in Immunology, John Wiley & Sons, Inc. (New York) (1992-2000)

Frank, Opthalmic Res 29:341-53 (19997)

Galvin et al., PNAS USA, 93:7894-7899 (1996)

Gennaro (ed.), Remington's Pharmaceutical Sciences, 18th Ed. (Mack Publishing Co.1990)

Gerwins et al., Crit Rev Oncol Hematol 34(3):185-94 (2000)

Grigoriadis et al, J Cell Biol 106(6):2139-51 (1988)

Harlow et al, Antibodies: A Laboratory Manual, CSHL (Cold Spring Harbor, NY) (1988)

Knappik et al., J. Mol. Biol., 296:57-86 (2000)

Kohfeldt et al., FEBS Lett. 414:557-561, 1997

Kohler and Milstein, Nature, 256(5517):495-497 (1975)

Liu et al, PNAS USA. 84(10):3439-3443 (1987)

Martin, Genes Dev. 12:1571-1586 (1998)

Meinkoth et al, Anal Biochem 138:267-284 (1984)

Meyers et al., Nature Genetics, 11:462-464 (1995)

Morrison et al., PNAS USA 81(21):6851-6855 (1984)

Muenke et al., Am. J. Hum. Genet., 60:555-564 (1997)

Neuberger et al, Nature 314(6008):268-270 (1985)

Ornitz et al, J Biol Chem 267:16305-16311 (1992)

Ornitz, Novartis Found Symp 232:63-76; discussion 76-80, 272-82 (2001)

Paques et al., Diabetes Metab. 23(2):125-30 (1997)

Queen et al., PNAS USA, 86:10029-10033 (1989)

Saito et al., Mol Cell Biol, 21(19):6387-94 (2001)

Sato et al., Ann N Y Acad Sci, 902:201-5; discussion 205-7 (2000)

Saltzman et al, Biophys. J. 55:163 (1989)

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning: A laboratory manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, USA.

Schell et al., Hum Mol Gen, 4:323-328 (1995)

Sherwood et al., Biotechnology, 10(11):1446-9 (1992)

Tavormina et al., Am. J. Hum. Genet., 64:722-731 (1999)

Vajo et al., Endocrine Reviews, 21(1):23-39 (2000)

Webster et al., Trends Genetics 13(5):178-182 (1997)

Yamaguchi et al., EMBO J. 18(16):4414-4423 (1999)

CLAIMS

1. A molecule comprising the antigen binding portion of an isolated antibody which has specific binding affinity for a receptor protein tyrosine kinase and which blocks constitutive activation of said receptor protein tyrosine kinase.
2. The molecule according to claim 1, wherein said molecule binds to the extracellular domain of the receptor protein tyrosine kinase.
3. The molecule according to claim 1 wherein the antibody binds the dimeric form of the receptor.
4. The molecule according to claim 1, wherein the receptor protein tyrosine kinase is selected from the group consisting of EGFR/ErbB1, ErbB2/HER2/Neu, ErbB/HER3, ErbB4/HER4, IGF-1R, PDGFR-α, PDGFR-β, CSF-1R, kit/SCFR, Flk2/FH3, Flk1/VEGFR1, Flk1/VEGFR2, Flt4/VEGFR3, FGFR1, FGFR2/K-SAM, FGFR3, FGFR4, TrkA, TrkC, HGFR, RON, EphA2, EphB2, EphB4, Axl, TIE/TIE1, Tek/TIE2, Ret, ROS, Alk, Ryk, DDR, LTK and MUSK, and heterodimeric combinations thereof.
5. The molecule according to claim 4, wherein said receptor protein tyrosine kinase is a fibroblast growth factor receptor (FGFR).
6. The molecule according to claim 5, wherein said FGFR is FGFR3.
7. A pharmaceutical composition, comprising, as an active ingredient, the molecule according to any one of claims 1 through 6 and a pharmaceutically acceptable carrier, excipient, or auxiliary agent.
8. A molecule comprising the antigen-binding portion of an antibody which has specific binding affinity for a fibroblast growth factor receptor (FGFR) and which blocks ligand-dependent activation of said FGFR.
9. The molecule according to claim 8, wherein said molecule binds to the extracellular domain of the FGFR.
10. The molecule according to claim 9, wherein the FGFR is FGFR3.
11. A pharmaceutical composition, comprising the molecule according to any one of claims 8-10 and a pharmaceutically acceptable carrier, excipient, or auxiliary agent.

12. A kit comprising a molecule of any one of claims 1-6 and 8-10 and at least one reagent suitable for detecting the presence of said molecule when bound to said receptor protein tyrosine kinase and instructions for use.

13. A method for treatment of bone and cartilage related disorders, comprising administering a therapeutically effective amount of the pharmaceutical composition according to claim 7 or 11 to a subject in need thereof.

14. The method according to claim 13 wherein the skeletal disorder is a skeletal dysplasia or a craniosynostosis disorder.

15. The method according to claim 14 wherein said craniosynostosis disorder is Muenke coronal craniosynostosis or Crouzon syndrome with acanthosis nigricans.

16. The method according to claim 13 wherein the skeletal dysplasia is selected from achondroplasia, thanatophoric dysplasia (TD), hypochondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN) dysplasia.

17. The method according to claim 16, wherein the skeletal dysplasia is achondroplasia.

18. The method according to claim 13 for treating or inhibiting a malignant cell proliferative disease or disorder associated with abnormal RPTK activity.

19. The method according to claim 18 wherein the malignant cell proliferative disease or disorder is a hematopoietic malignancy.

20. The method according to claim 19 wherein the hematopoietic malignancy is multiple myeloma.

21. The method according to claim 18 for the treatment or inhibition of solid tumors.

22. The method according to claim 21 wherein the solid tumors are selected from mammary, colon, cervical, bladder, colorectal, chondrosarcoma or osteosarcoma.

23. The method according to claim 18 for treating or inhibiting tumor formation, primary tumors, tumor progression or tumor metastasis.

24. The method according to claim 23 wherein tumor progression is the progression of transitional cell carcinoma.

25. The method according to claim 19 wherein the disorder is associated with the action of a constitutively activated receptor protein tyrosine kinase, and wherein the administered pharmaceutical composition is the pharmaceutical composition according to claim 7.

26. The method according to claim 19, wherein the disorder is associated with ligand-dependent activation of a receptor protein tyrosine kinase, and wherein the administered pharmaceutical composition is the pharmaceutical composition according to claim 11.

27. The method according to claim 18 for treatment of hyperproliferative diseases and disorders associated with ligand dependent fibroblast growth factor receptor signaling.

28. The method according to claim 27 wherein the hyperproliferative diseases and disorders are vision disorders such as neovascular glaucoma, macular degeneration and proliferative retinopathy including diabetic retinopathy.

29. The method according to claim 27 wherein the hyperproliferative diseases are non-neoplastic angiogenic pathologic conditions such as hemangiomas, angiofibromas and psoriasis

30. The method according to claim 18, wherein the disorder is associated with constitutive activation of a receptor protein tyrosine kinase, and wherein the administered pharmaceutical composition is the pharmaceutical composition according to claim 7.

31. The method according to claim 18, wherein the disorder is associated with ligand-dependent activation of a receptor protein tyrosine kinase, and wherein the administered pharmaceutical composition is the pharmaceutical composition according to claim 11.

32. A method for treating or inhibiting a cell proliferative disease or disorder, comprising administering a therapeutically effective amount of the pharmaceutical composition according to claim 7 or 11 to a subject in need thereof.

33. The method according to claim 19, wherein the cell proliferative disease or disorder is tumor progression.

34. The method according to claim 20, wherein the tumor progression is the progression of transitional cell carcinoma.

35. The method according to claim 20, wherein the tumor progression is the progression of osteo or chondrosarcoma.

36. The method according to claim 20, wherein the tumor progression is the progression of multiple myeloma.

37. The method according to claim 19 wherein the receptor protein tyrosine kinase is FGFR3 and the tumor progression is the progression of mammary carcinoma.

38. A method for screening a molecule comprising the antigen-binding portion of an antibody which blocks ligand-dependent activation of a receptor protein tyrosine kinase, comprising:
screening a library of antibody fragments for binding to a dimeric form of a receptor protein tyrosine kinase;
identifying an antibody fragment which binds to the dimeric form of the receptor protein tyrosine kinase as a candidate molecule for blocking ligand-dependent activation of the receptor protein tyrosine kinase;
and determining whether or not the candidate molecule can block ligand-dependent activation of the receptor protein tyrosine kinase in a cell.

39. The method according to claim 38, wherein the receptor protein tyrosine kinase is a fibroblast growth factor receptor

40. The method according to claim 39, wherein the fibroblast growth factor receptor is FGFR3.

41. A molecule according to claim 1 comprising V_H-CDR3 and V_L-CDR3 regions, selected from the group consisting of SEQ ID NO: 8 and 9; SEQ ID NO: 12 and 13; and SEQ ID NO: 24 and 25.

42. The molecule according to claim 41, comprising V_L region and V_H regions, selected from the group consisting of SEQ ID NO: 92 and 103; SEQ ID NO: 94 and 105 and SEQ ID NO: 102 and 113.

43. A pharmaceutical composition, comprising, as an active ingredient, the molecule according to any one of claims 41 or 42 and a pharmaceutically acceptable carrier, excipient, or auxiliary agent.

44. An isolated nucleic acid molecule, comprising a sequence selected from SEQ ID NO: 30, 31, 34, 35, 50 or 51 or a nucleotide sequence hybridizing under high stringency conditions thereto.

45. An isolated nucleic acid molecule, comprising a sequence selected from SEQ ID NO: 74, 75, 76, 84, 89 or 91 and 87or a nucleotide sequence hybridizing under high stringency conditions thereto.

46. The isolated nucleic acid molecule of claim 44, comprising nucleotides encoding a V_L-CDR3 DNA region and a V_H-CDR3 DNA region, respectively, selected from the group consisting of SEQ ID NO: 30 and 31; SEQ ID NO:34 and 35; SEQ ID NO: 50 and 51.

47. The isolated nucleic acid molecule of claim 45 comprising nucleotides encoding a V_L region and a V_H region, respectively, selected from the group consisting of SEQ ID NO: 74 and 84 ; SEQ ID NO:75 and 89; and SEQ ID NO: 76 and 91.

48. A vector comprising a nucleic acid molecule according to claim 46 or 47.

49. The vector according to claim 48 wherein the vector is an expression vector.

50. The host cell transformed with the vector according to claim 48 or 49.

51. A molecule according to claim 8 comprising the combination of V_H-CDR3 and V_L-CDR3 amino acid sequences selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:11; SEQ ID NO:14 and SEQ ID NO:15; SEQ ID NO:16 and SEQ ID NO:17; SEQ ID NO:18 and SEQ ID NO:19; SEQ ID NO:20 and SEQ ID NO:21; SEQ ID NO:22 and SEQ ID NO:23, SEQ ID NO:26 and SEQ ID NO:27 or SEQ ID NO:28 and SEQ ID NO:29.

52. The molecule according to claim 51, comprising a V_L region and a V_H region, respectively, selected from the group consisting of respectively, selected from the group consisting of SEQ ID NO: 92 and 103; SEQ ID NO: 93 and 104; SEQ ID NO: 94 and 105; SEQ ID NO:95 and 106; SEQ ID NO: 96 and 107 ; SEQ ID NO: 97 and 108; SEQ ID NO:98 and 109; SEQ ID NO: 99 and 110; SEQ ID NO: 100 and 111; SEQ ID NO: 101 and 112; and SEQ ID NO:102 and 113.

53. A pharmaceutical composition, comprising the molecule according to any one of claims 51-52 and a pharmaceutically acceptable carrier, excipient, or auxiliary agent.

54. An isolated nucleic acid molecule, comprising SEQ ID NO: NO:32, 33, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 48, and 49 or a nucleotide sequence hybridizing under high stringency conditions thereto.

55. An isolated nucleic acid molecule, comprising SEQ ID NO: 62, 64, 65, 67, 69, 70, 76, 78, 79, 80, 83, 85, 86, and 87or a nucleotide sequence hybridizing under high stringency conditions thereto.

56. An isolated nucleic acid molecule, comprising nucleotides encoding a V_L-CDR3 DNA region and a V_H-CDR3 DNA region, respectively, selected from the group consisting of SEQ ID NO: 32 and 33; SEQ ID NO:36 and 37; SEQ ID NO: 38 and 39, SEQ ID NO:40 and 41, SEQ ID NO: 42 and 43, SEQ ID NO: 44 and 45, SEQ ID NO: 48 and 49.

57. An isolated nucleic acid molecule, comprising nucleotides encoding a V_L region and a V_H region, respectively, selected from the group consisting of SEQ ID NO: 70 and 85; SEQ ID NO:67 and 78; SEQ ID NO:64 and 79; SEQ ID NO:71 and 86; SEQ ID NO:62 and 80; SEQ ID NO:65 and 87; SEQ ID NO:69 and 83.

58. A vector comprising a nucleic acid molecule according to claim 56 or 57.

59. The vector according to claim 58 which is an expression vector.

60. The host cell transformed with the vector according claim 58 or 59.

61. A method for treatment of bone and cartilage related disorders, comprising administering a therapeutically effective amount of the pharmaceutical composition according to claim 43 or 53 to a subject in need thereof.

62. The method according to claim 61 wherein the skeletal disorder is a skeletal dysplasia or a craniosynostosis disorder.

63. The method according to claim 62 wherein said craniosynostosis disorder is Muenke coronal craniosynostosis or Crouzon syndrome with acanthosis nigricans.

64. The method according to claim 63 wherein the skeletal dysplasia is selected from achondroplasia, thanatophoric dysplasia (TD), hypochondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN) dysplasia.

65. The method according to claim 64, wherein the skeletal dysplasia is achondroplasia.

66. The method according to claim 61 for treating or inhibiting a malignant cell proliferative disease or disorder associated with abnormal RPTK activity.

67. The method according to claim 66 wherein the malignant cell proliferative disease or disorder is a hematopoietic malignancy.

68. The method according to claim 67 wherein the hematopoietic malignancy is multiple myeloma.

69. The method according to claim 61 for the treatment or inhibition of solid tumors.

70. The method according to claim 69 wherein the solid tumors are selected from mammary, colon, cervical, bladder, colorectal, chondrosarcoma or osteosarcoma.

71. The method according to claim 61 for treating or inhibiting tumor formation, primary tumors, tumor progression or tumor metastasis.

72. The method according to claim 71 wherein tumor progression is the progression of transitional cell carcinoma.

73. The method according to claim 61 wherein the disorder is associated with the action of a constitutively activated receptor protein tyrosine kinase, and wherein the administered pharmaceutical composition is the pharmaceutical composition according to claim 43.

74. The method according to claim 61, wherein the disorder is associated with ligand-dependent activation of a receptor protein tyrosine kinase, and wherein the administered pharmaceutical composition is the pharmaceutical composition according to claim 53.

75. The method according to claim 74 for treatment of hyperproliferative diseases and disorders associated with ligand dependent fibroblast growth factor receptor signaling.

76. The method according to claim 75 wherein the hyperproliferative diseases and disorders are vision disorders such as neovascular glaucoma, macular degeneration and proliferative retinopathy including diabetic retinopathy.

77. The method according to claim 75 wherein the hyperproliferative diseases are non-neoplastic angiogenic pathologic conditions such as hemangiomas, angiofibromas and psoriasis

78. The method according to claim 73, wherein the disorder is associated with constitutive activation of a receptor protein tyrosine kinase, and wherein the administered pharmaceutical composition is the pharmaceutical composition according to claim 43.

79. The method according to claim 74, wherein the disorder is associated with ligand-dependent activation of a receptor protein tyrosine kinase, and wherein the administered pharmaceutical composition is the pharmaceutical composition according to claim 53.

80. A method for treating or inhibiting a cell proliferative disease or disorder, comprising administering a therapeutically effective amount of the pharmaceutical composition according to claim 43 or 53 to a subject in need thereof.

81. The method according to claim 80, wherein the cell proliferative disease or disorder is tumor progression.

82. The method according to claim 81, wherein the tumor progression is the progression of transitional cell carcinoma.

83. The method according to claim 81, wherein the tumor progression is the progression of osteo or chondrosarcoma.

84. The method according to claim 81, wherein the tumor progression is the progression of multiple myeloma.

85. The method according to claim 81 wherein the receptor protein tyrosine kinase is FGFR3 and the tumor progression is the progression of mammary carcinoma.

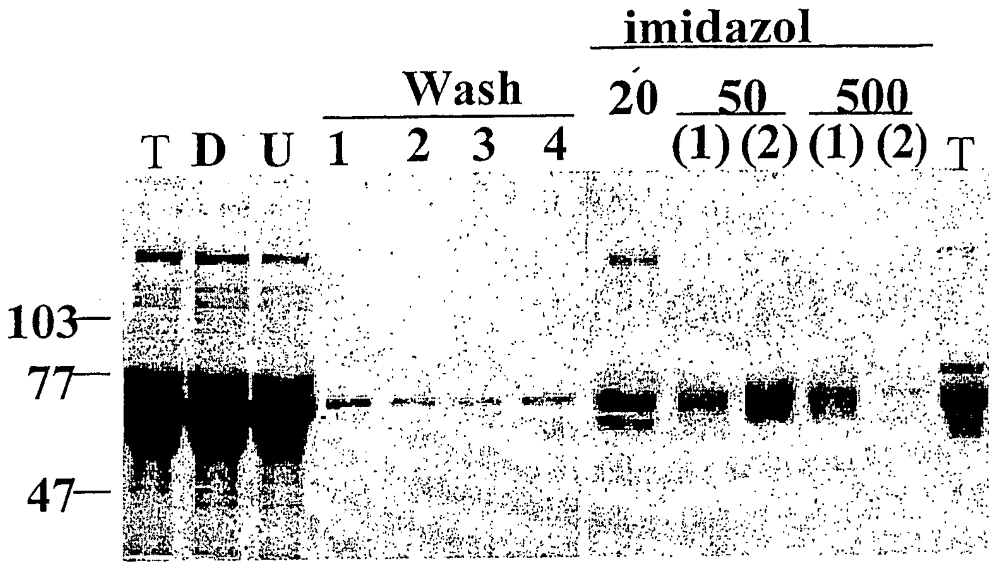
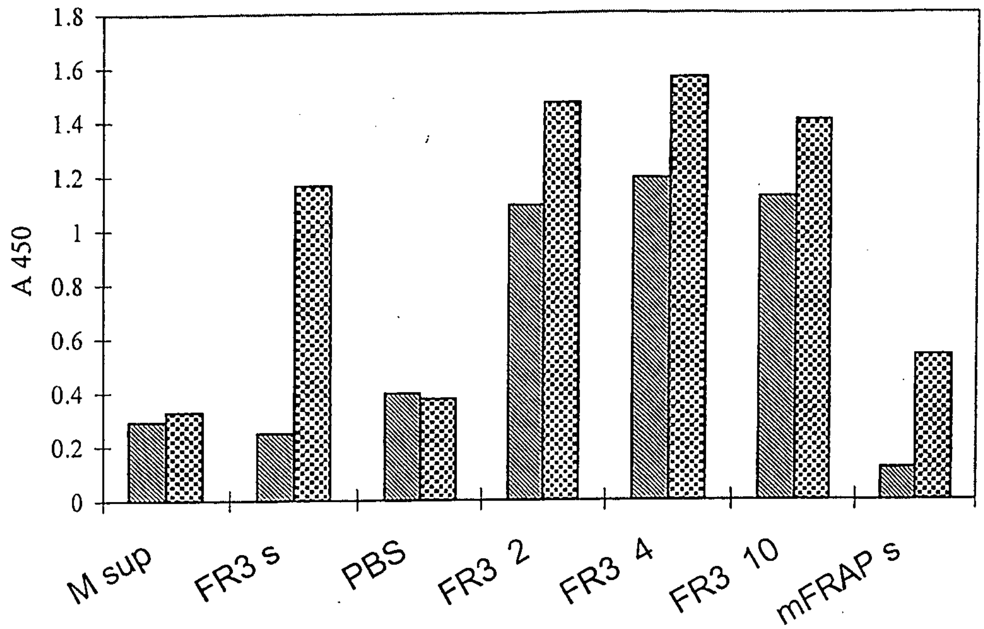


Figure 1

Figure 2



1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
A 450
M sup
FR3 s
PBS
FR3 2
FR3 4
FR3 10
mFRAP s

Figure 3

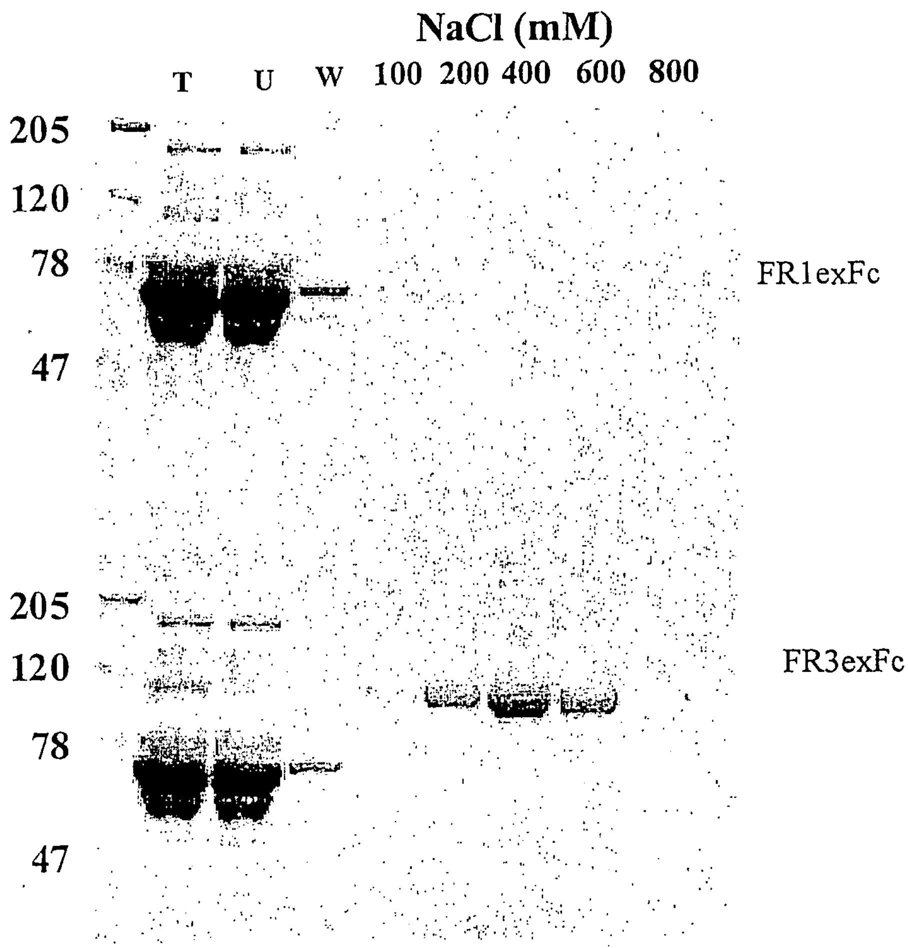

NaCl (mM)
T U W 100 200 400 600 800
205
120
78
47
FR1exFc
205
120
78
47
FR3exFc

Figure 4

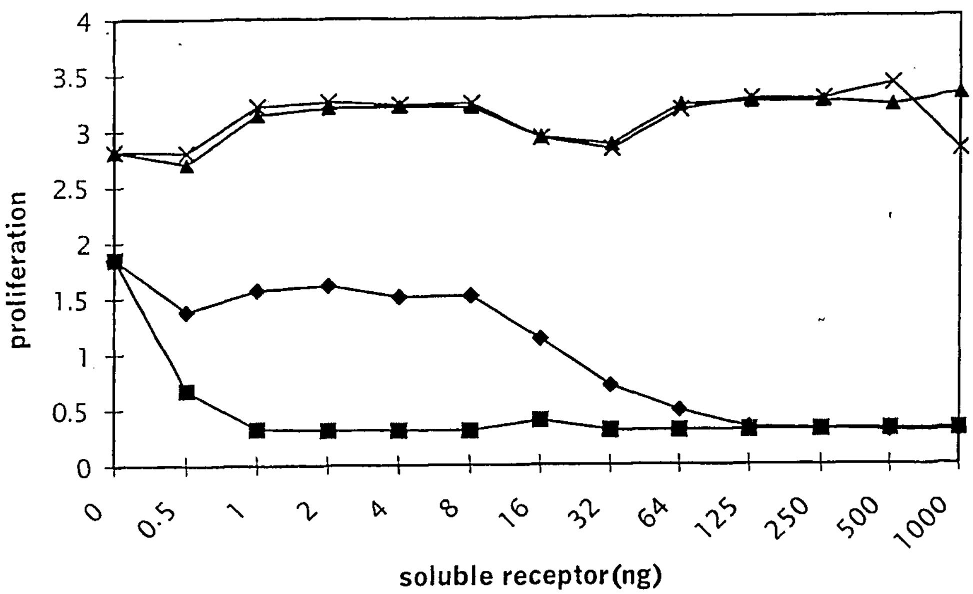

4
3.5
3
2.5
2
1.5
1
0.5
0
proliferation
0
0.5
1
2
4
8
16
32
64
125
250
500
1000
soluble receptor(ng)

Figure 5

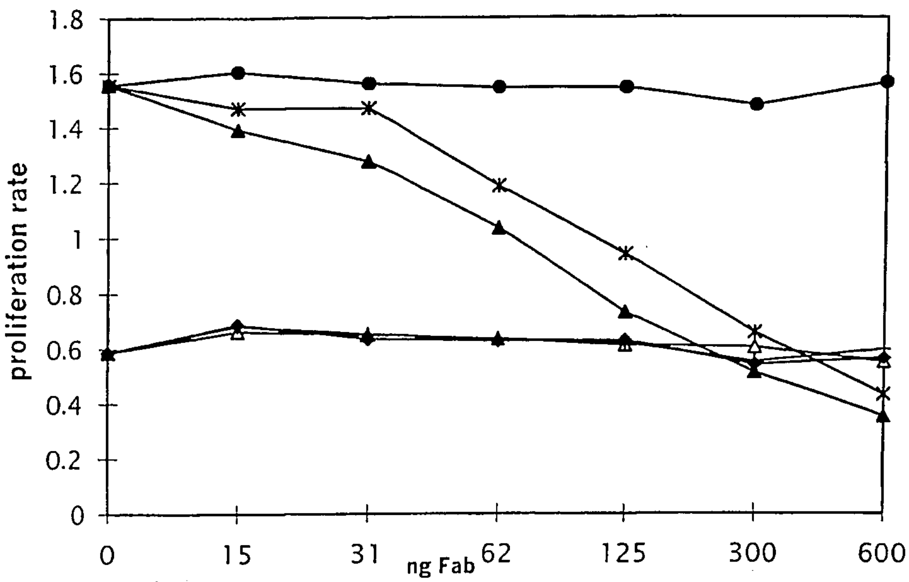

1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
proliferation rate
0
15
31
ng Fab
62
125
300
600

Figure 6

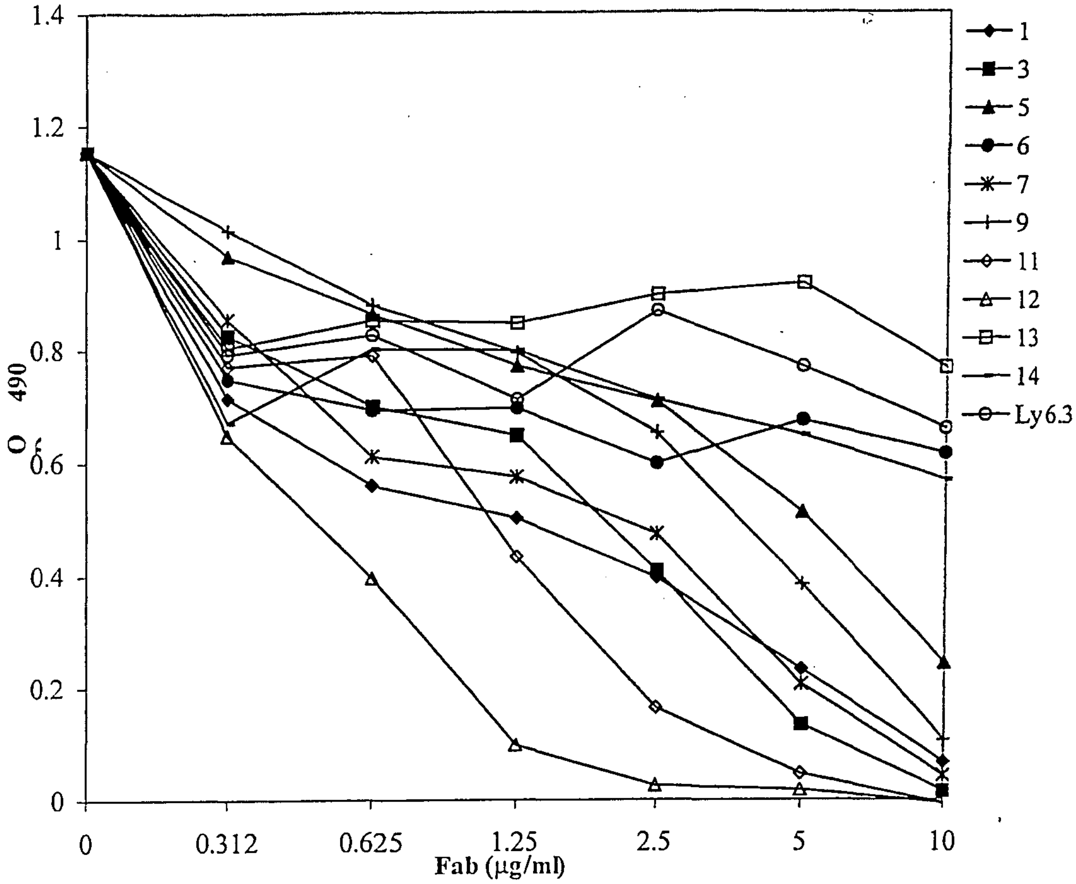

1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD 490
0
0.312
0.625
1.25
2.5
5
10
Fab (μg/ml)
1
3
5
6
7
9
11
12
13
14
Ly6.3

Figure 7A

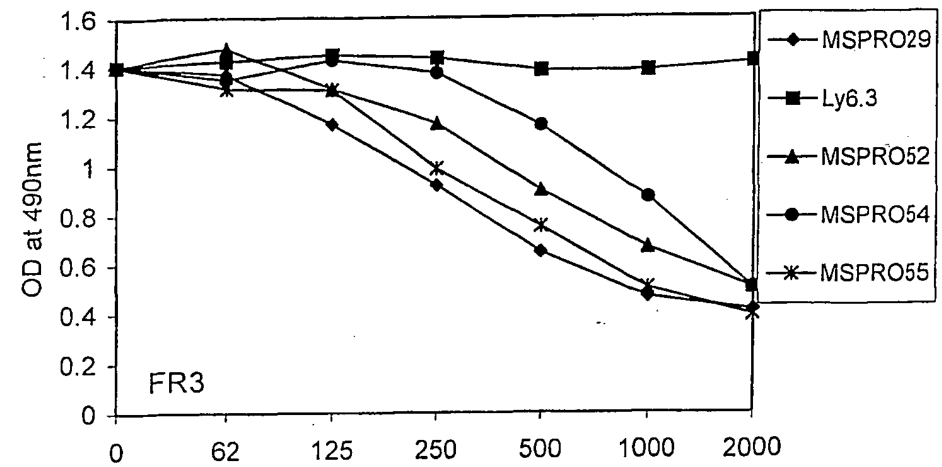


Figure 7B

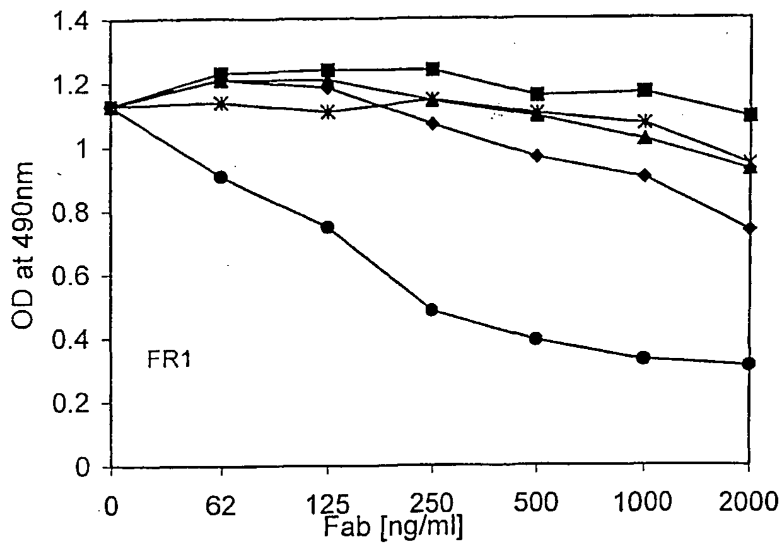


Figure 8A

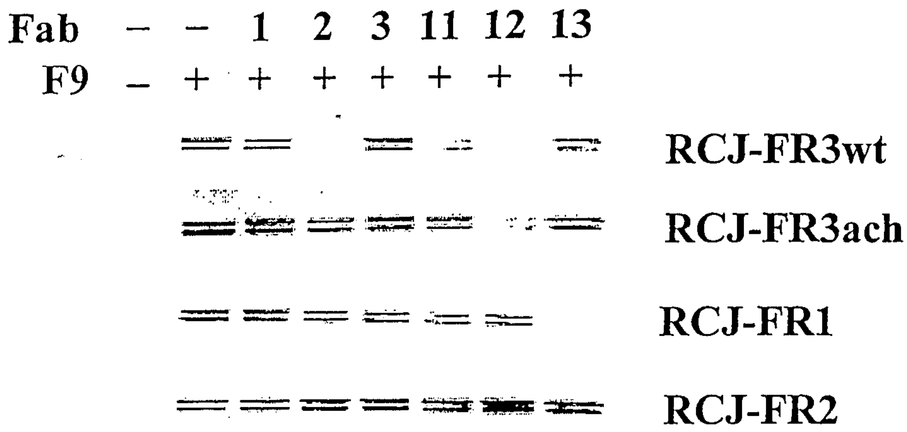
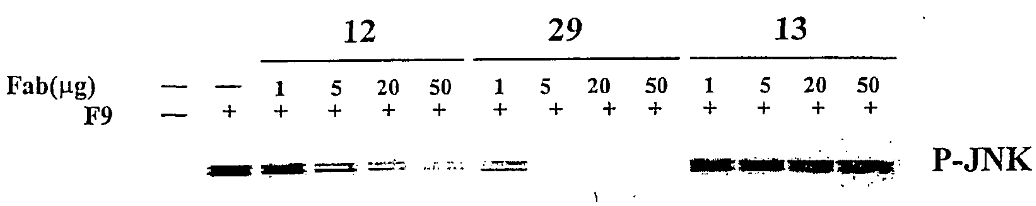


Figure 8B



SUBSTITUTE SHEET (RULE 26)

FIGURE 9A

M14

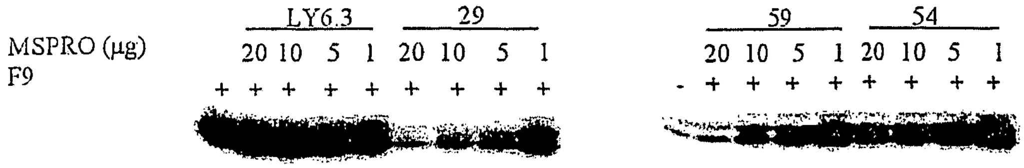


FIGURE 9B

W11

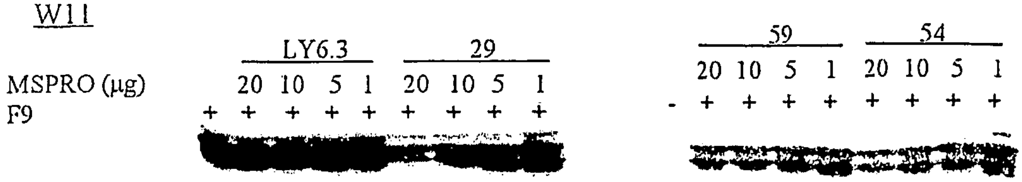


FIGURE 9C

R1-1

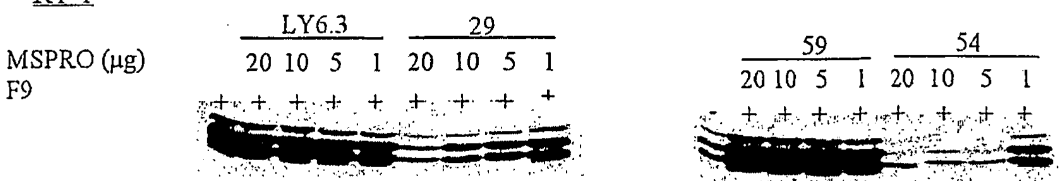


FIGURE 9D

R2-2

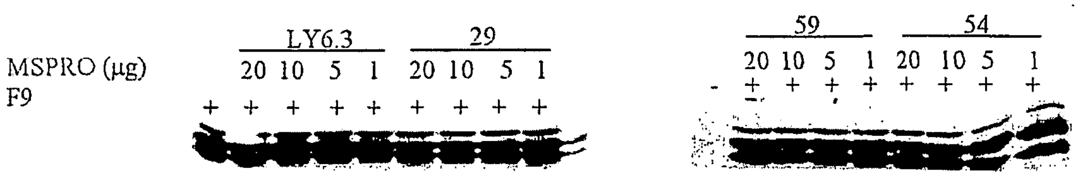


Figure 10

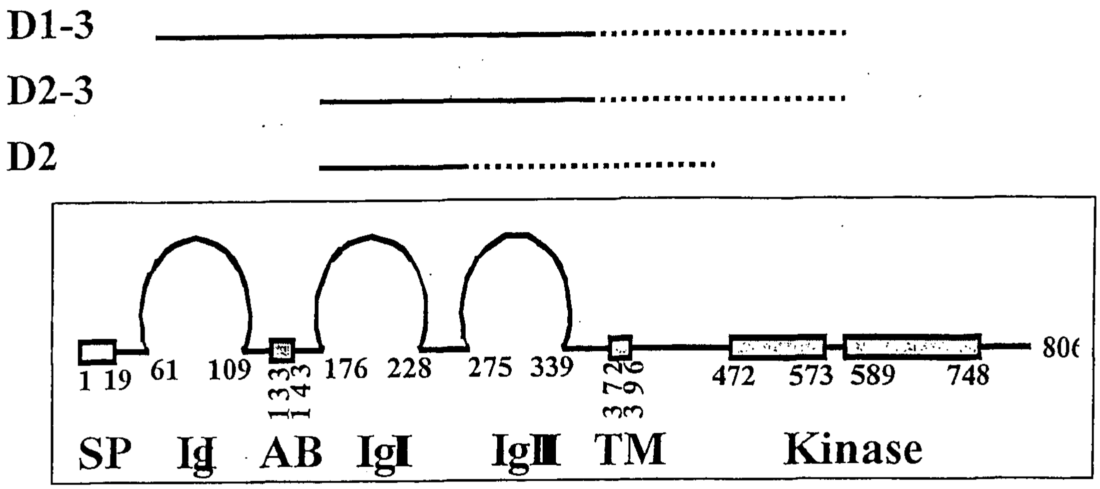
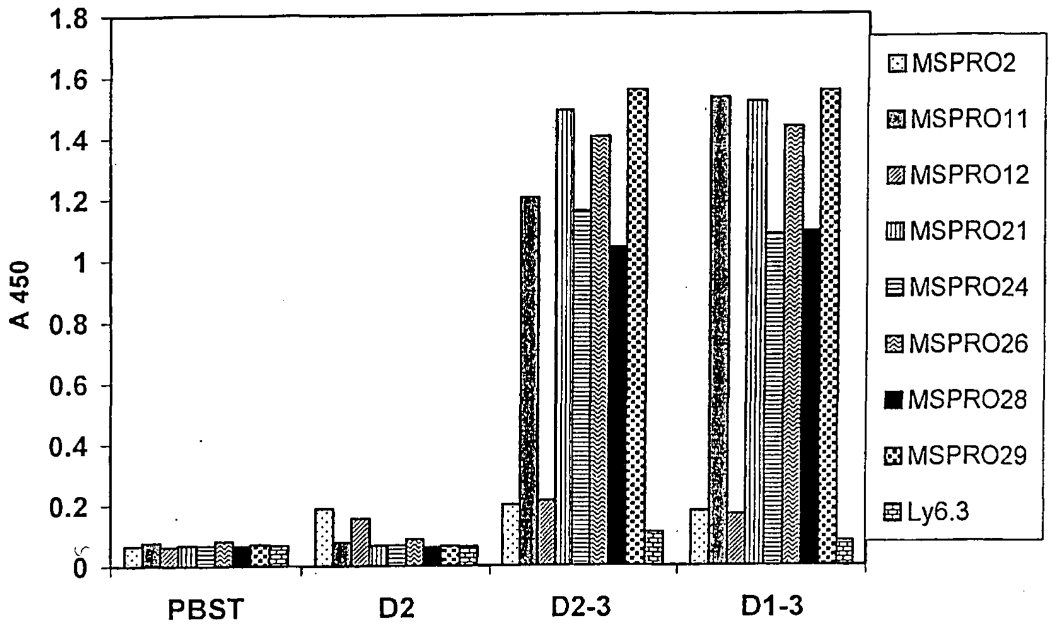

D1-3
D2-3
D2
1 19
61
109
133
143
176
228
275
339
372
396
472
573
589
748
SP
Ig
AB
IgI
IgII
TM
Kinase

Figure 11


1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
A 450
PBST
D2
D2-3
D1-3
MSPRO2
MSPRO11
MSPRO12
MSPRO21
MSPRO24
MSPRO26
MSPRO28
MSPRO29
Ly6.3

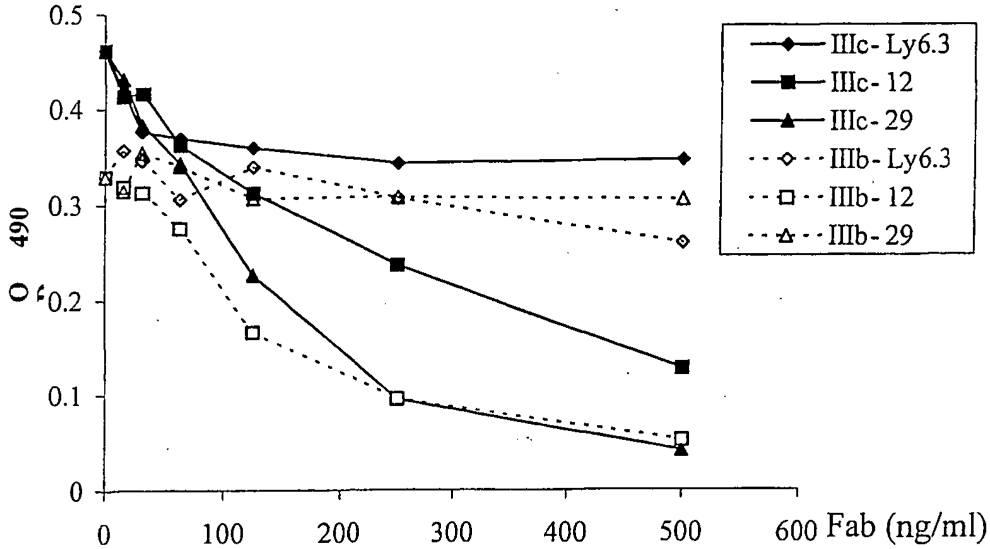

0.5
0.4
0.3
0.2
0.1
0
O 490
0
100
200
300
400
500
600
Fab (ng/ml)
IIIc- Ly6.3
IIIc- 12
IIIc- 29
IIIb- Ly6.3
IIIb- 12
IIIb- 29

Figure 12

Figure 13A

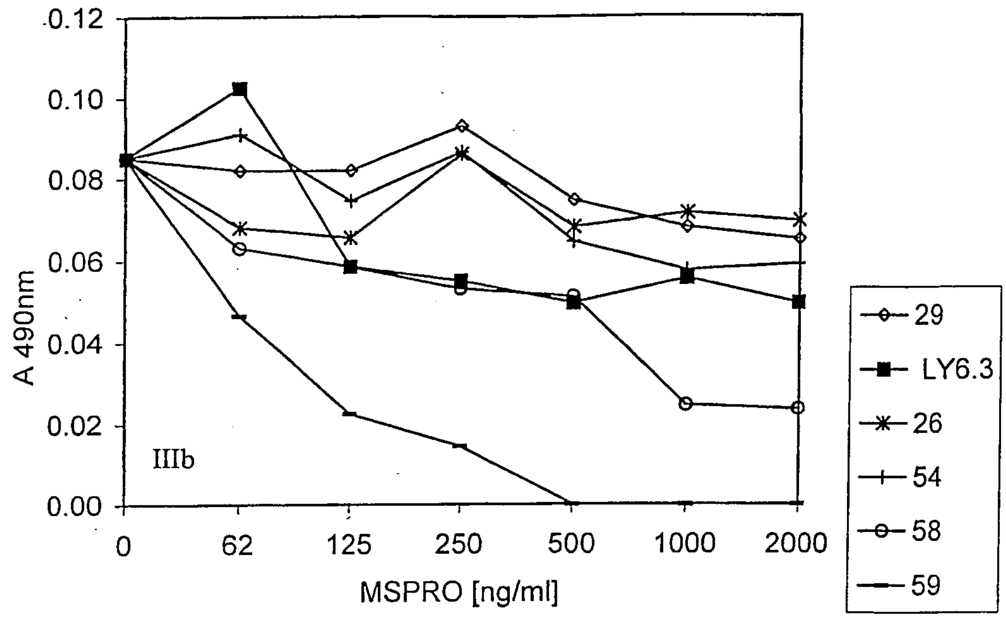


Figure 13B

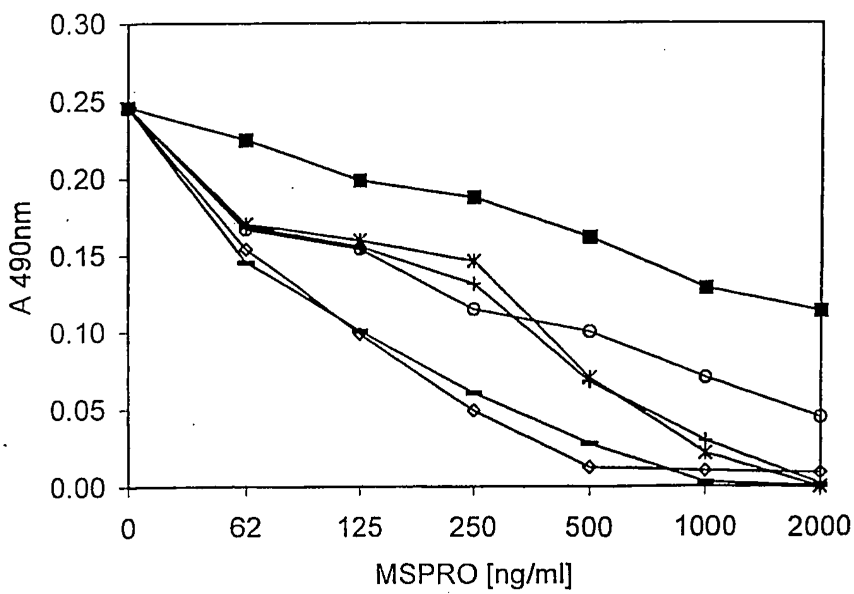


Figure 14

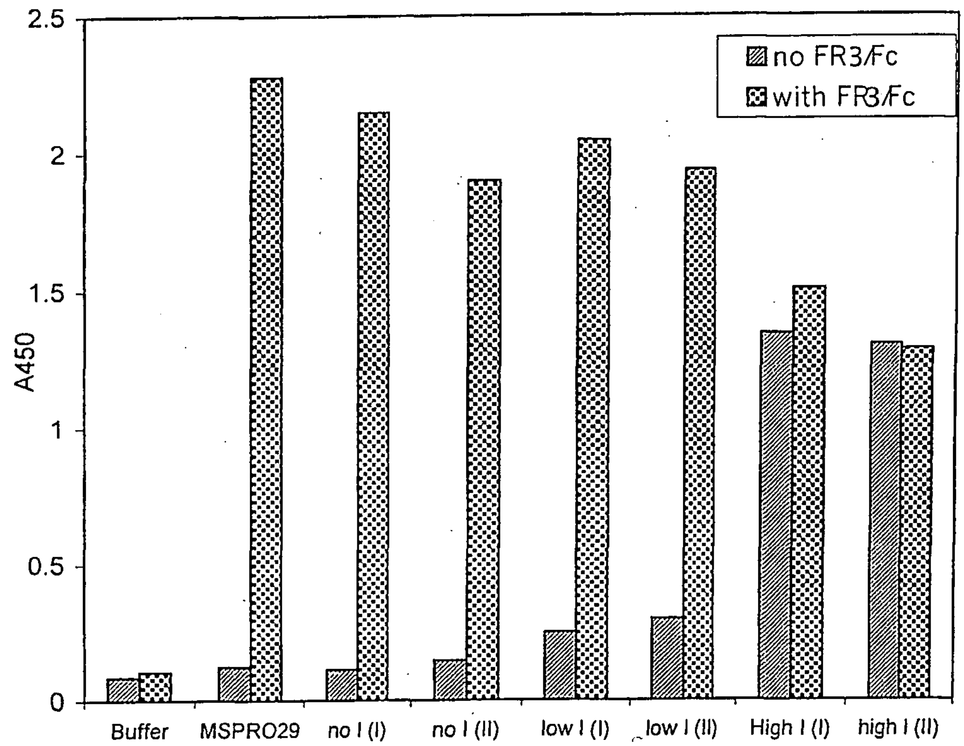

no FR3/Fc
with FR3/Fc
2.5
2
1.5
1
0.5
0
A450
Buffer
MSPRO29
no I (I)
no I (II)
low I (I)
low I (II)
High I (I)
high I (II)

Figure 15

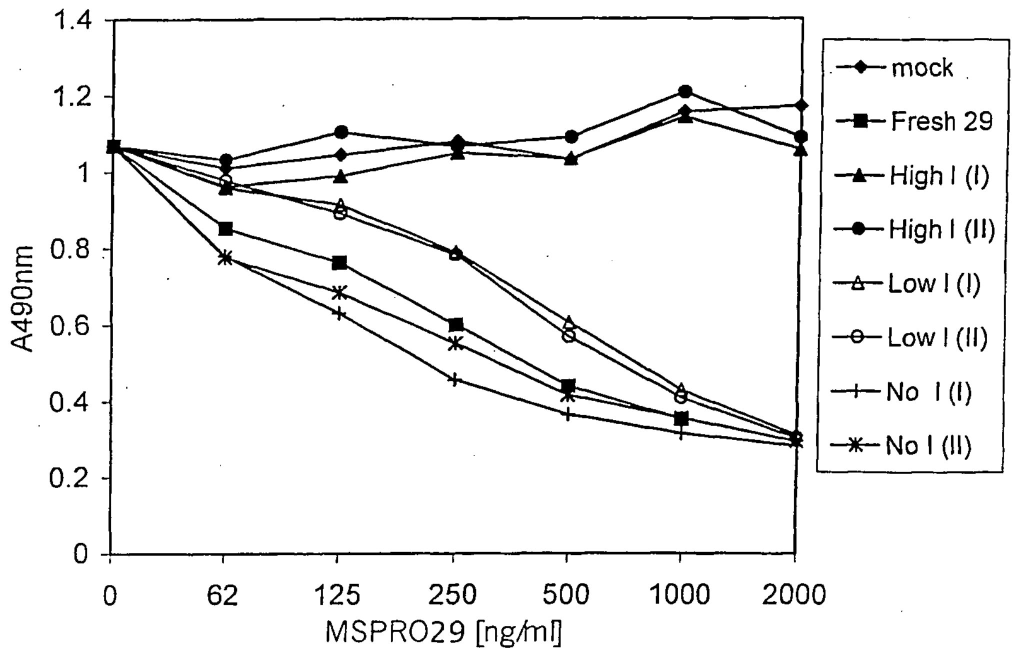

1.4
1.2
1
0.8
0.6
0.4
0.2
0
A490nm
0
62
125
250
500
1000
2000
MSPRO29 [ng/ml]
mock
Fresh 29
High I (I)
High I (II)
Low I (I)
Low I (II)
No I (I)
No I (II)

Figure 16

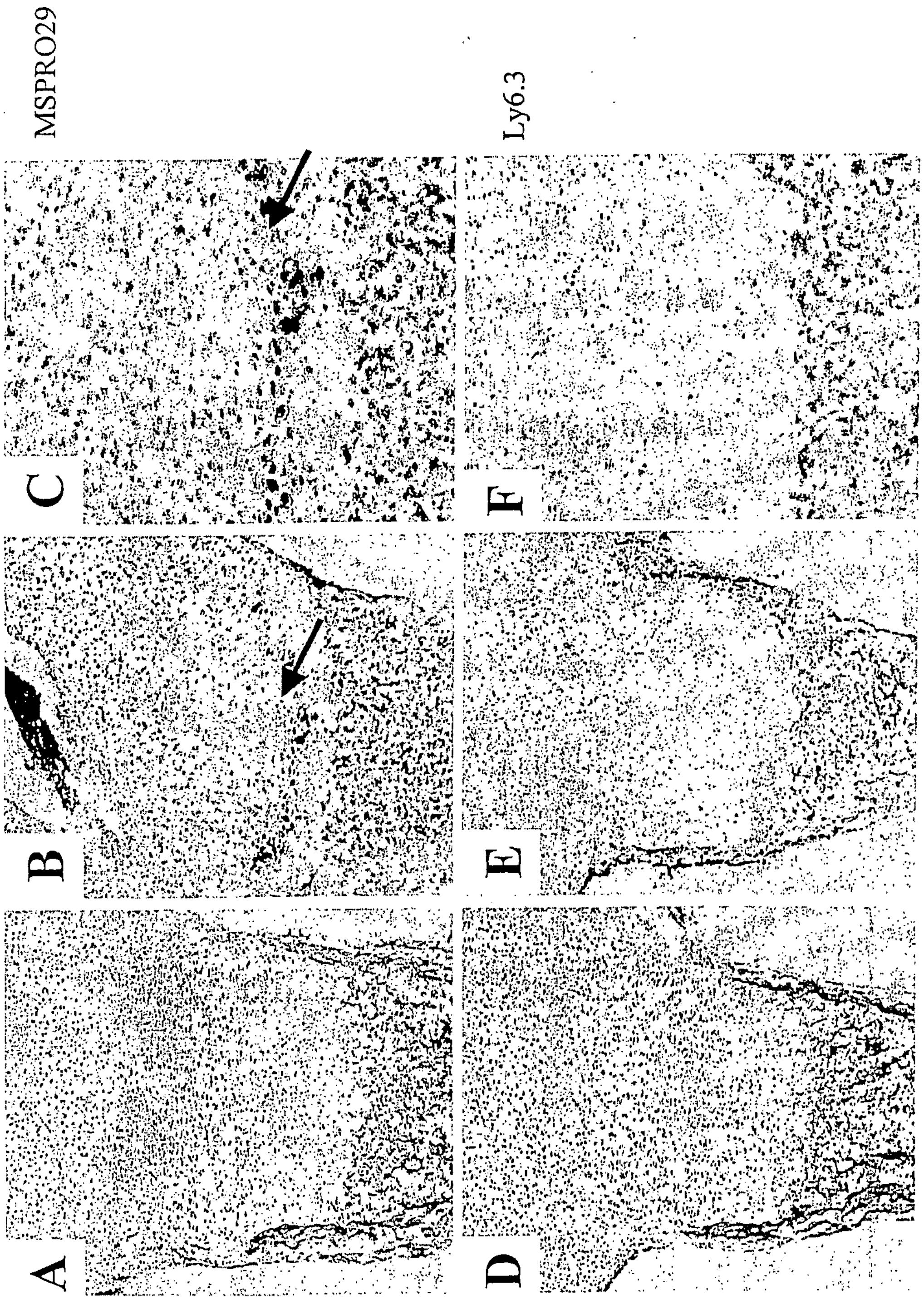


Figure 17A

Figure 17B

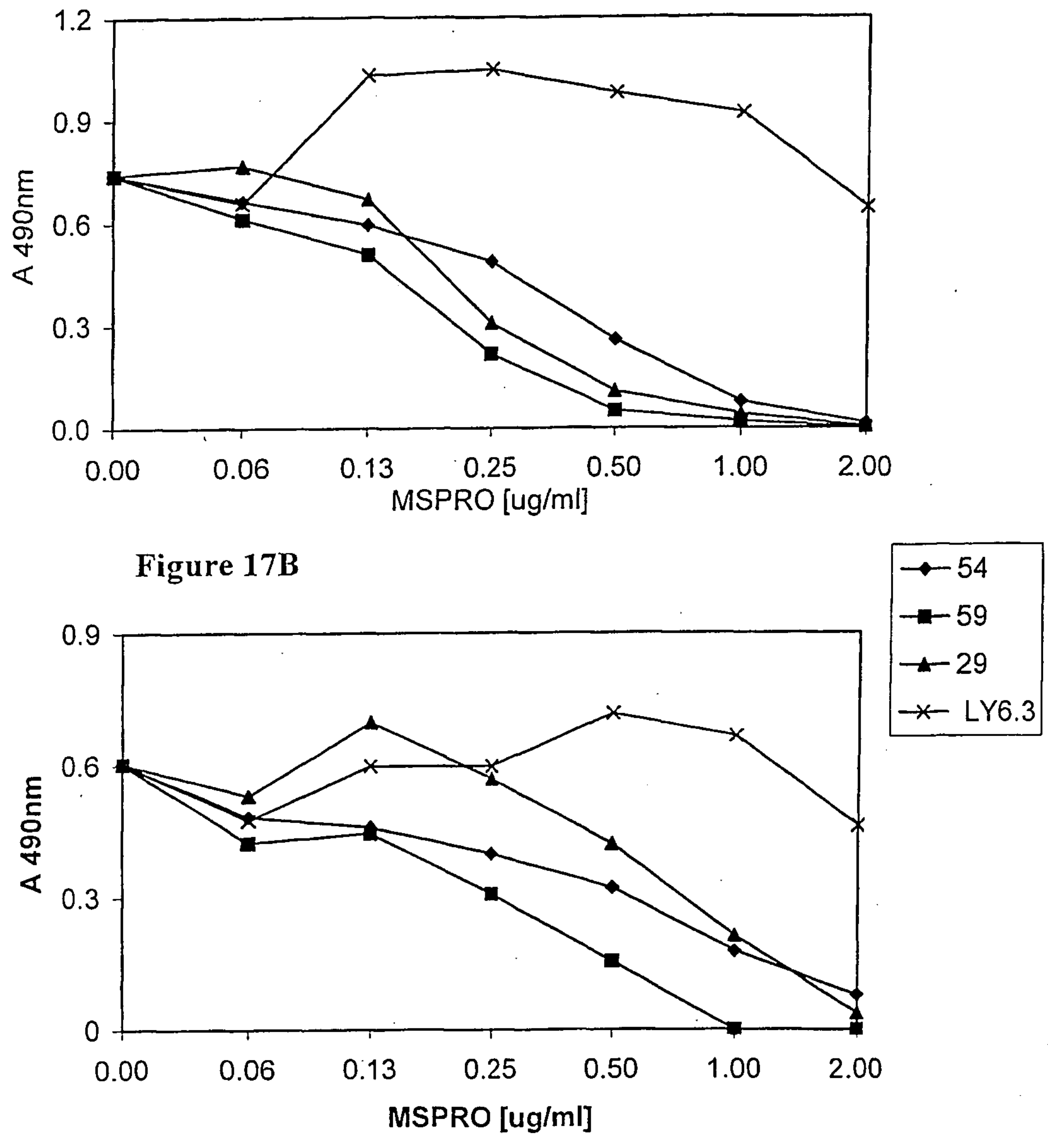


Figure 18A

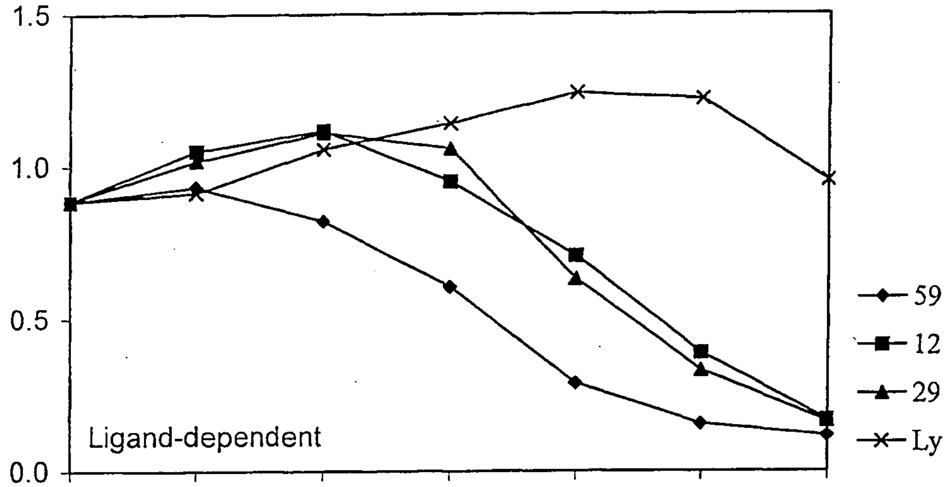


Figure 18B

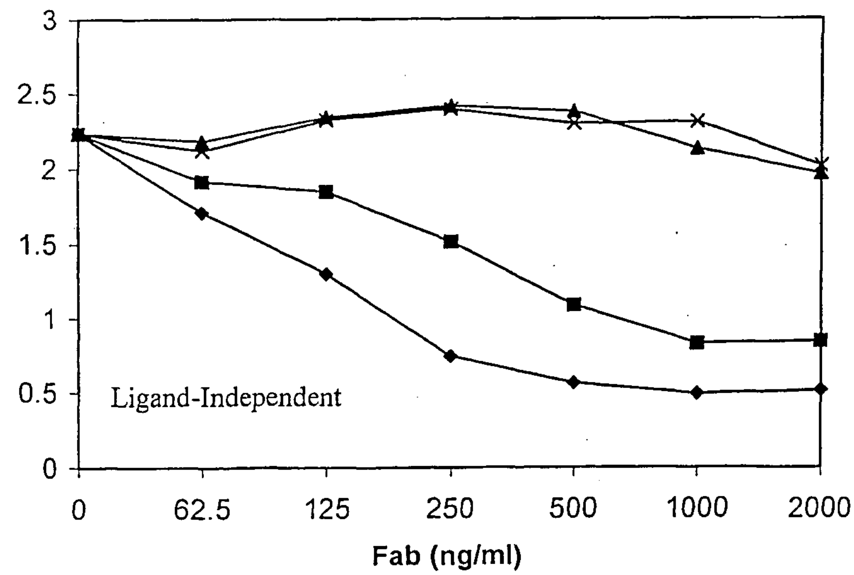


Figure 19

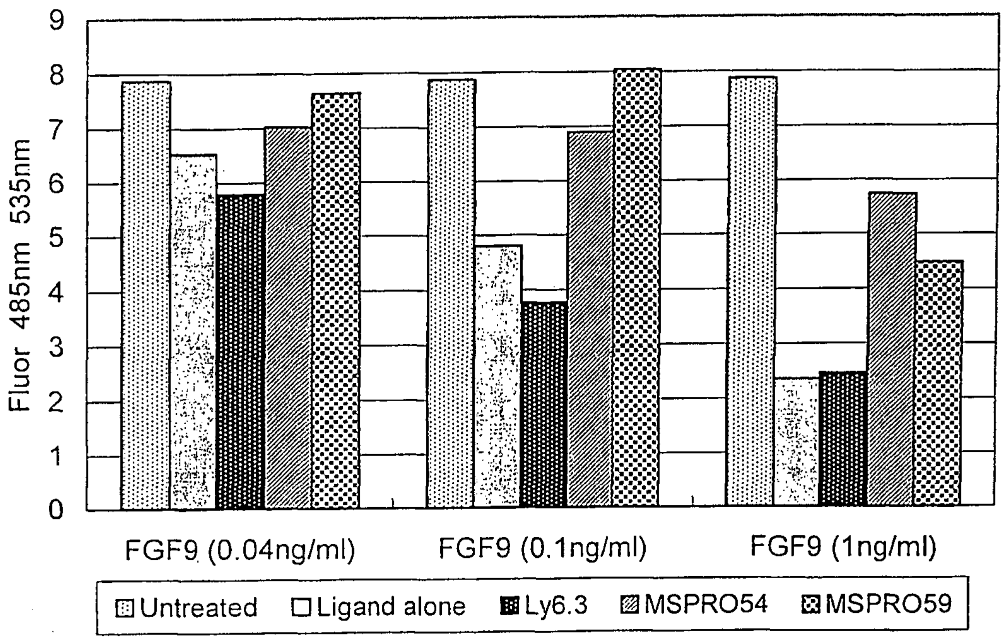
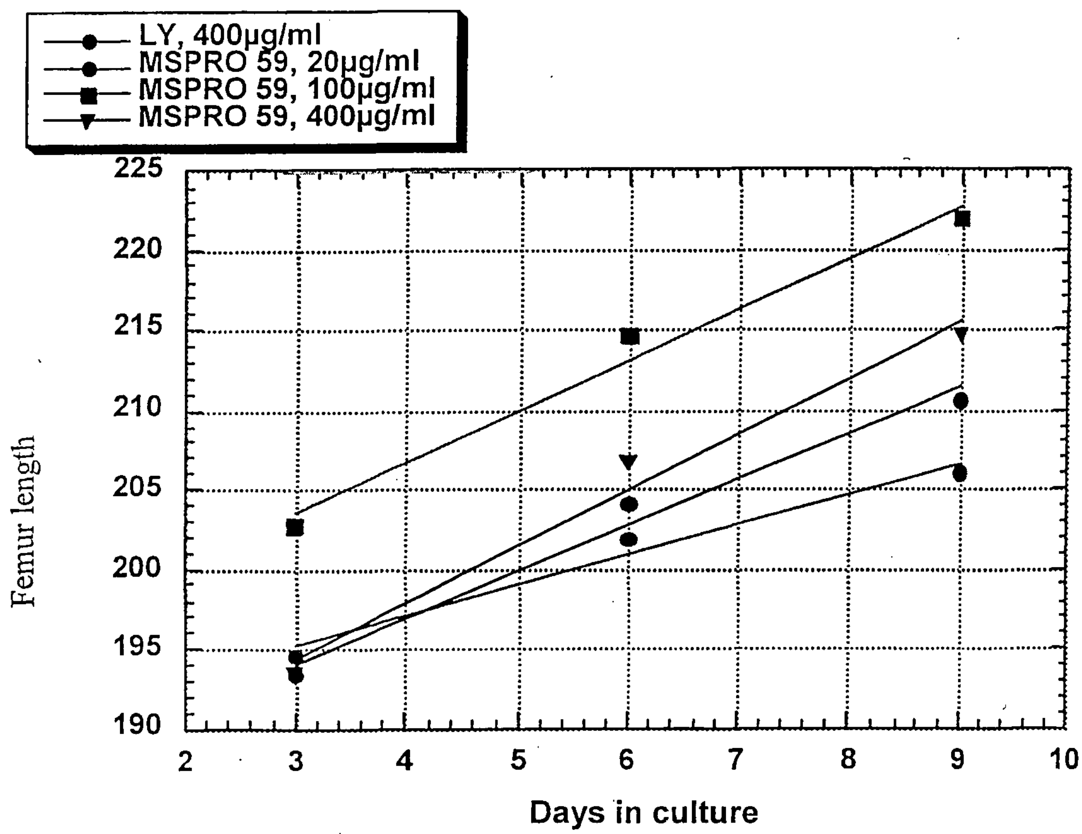

9
8
7
6
5
4
3
2
1
0
Fluor 485nm 535nm
FGF9 (0.04ng/ml)
FGF9 (0.1ng/ml)
FGF9 (1ng/ml)
Untreated
Ligand alone
Ly6.3
MSPRO54
MSPRO59

Figure 20



LY, 400µg/ml
MSPRO 59, 20µg/ml
MSPRO 59, 100µg/ml
MSPRO 59, 400µg/ml
225
220
215
210
205
200
195
190
Femur length
2
3
4
5
6
7
8
9
10
Days in culture

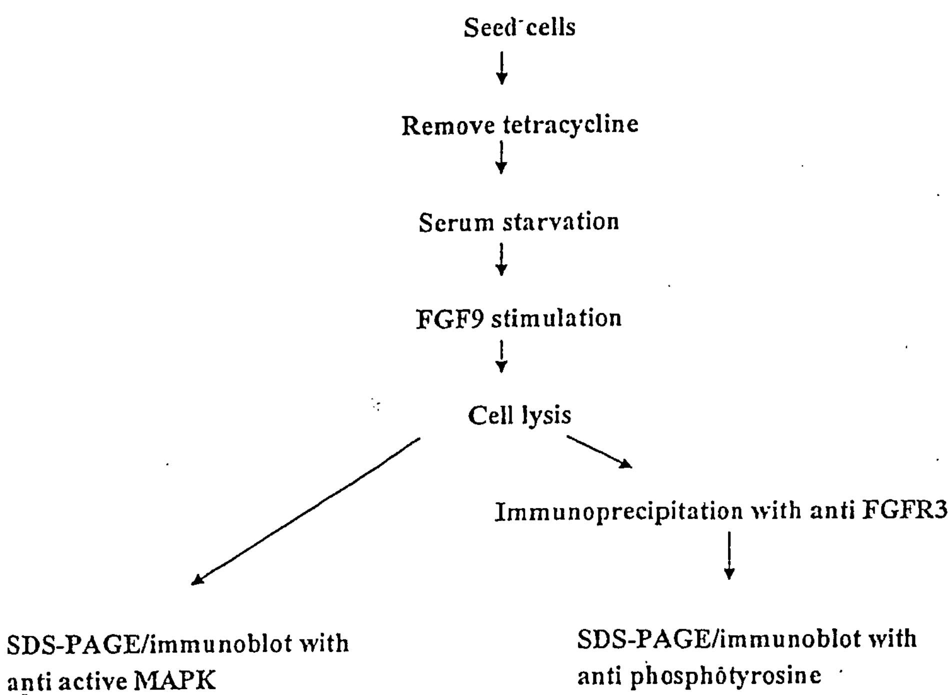

Seed cells
Remove tetracycline
Serum starvation
FGF9 stimulation
Cell lysis
Immunoprecipitation with anti FGFR3
SDS-PAGE/immunoblot with anti active MAPK
SDS-PAGE/immunoblot with anti phosphotyrosine

FIG.21

Figure 22

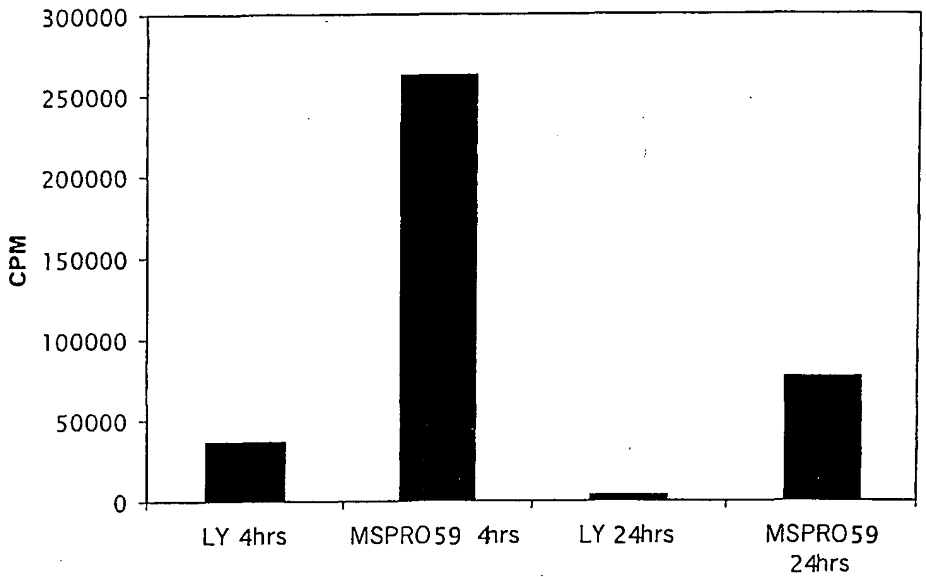

300000
250000
200000
150000
100000
50000
0
CPM
LY 4hrs
MSPRO59 4hrs
LY 24hrs
MSPRO59 24hrs

Figure 23

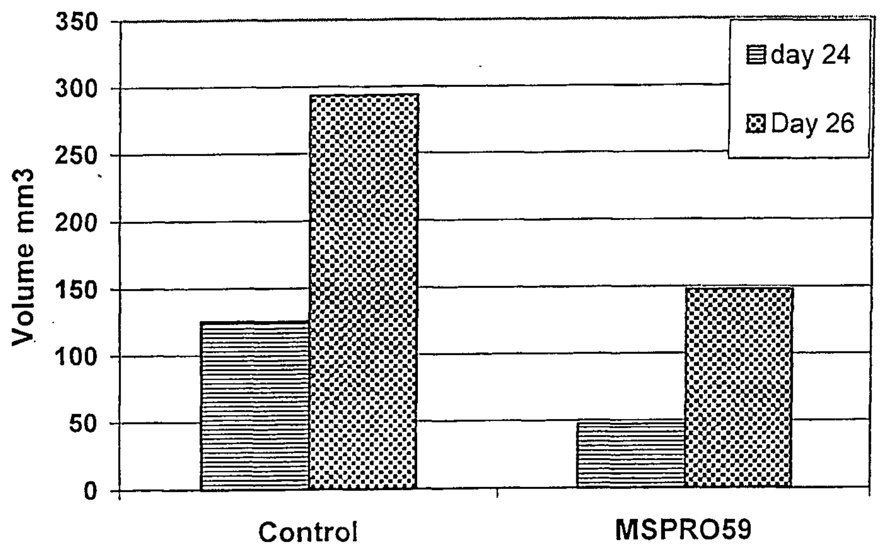
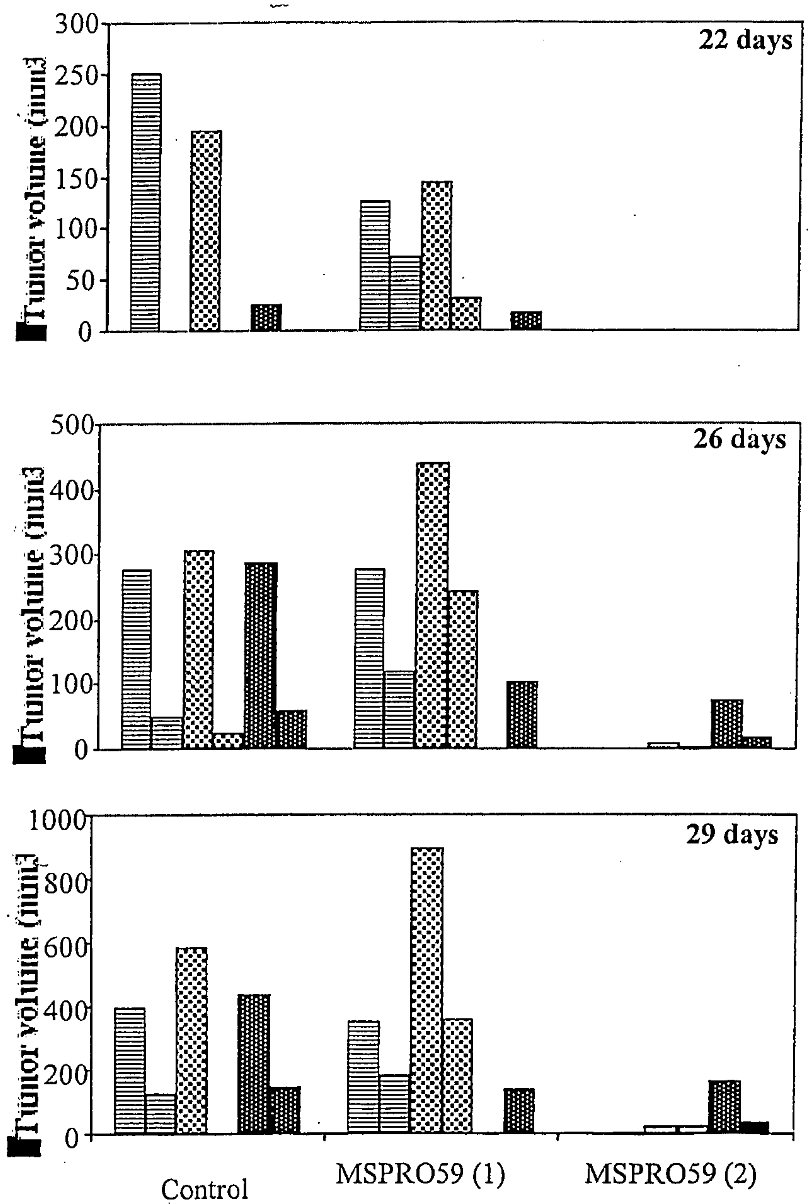

350
300
250
200
150
100
50
0
Volume mm3
day 24
Day 26
Control
MSPRO59

Figure 24



Tumor volume (mm3)
22 days
300
250
200
150
100
50
0
26 days
500
400
300
200
100
0
29 days
1000
800
600
400
200
0
Control
MSPRO59 (1)
MSPRO59 (2)

Figure 25A

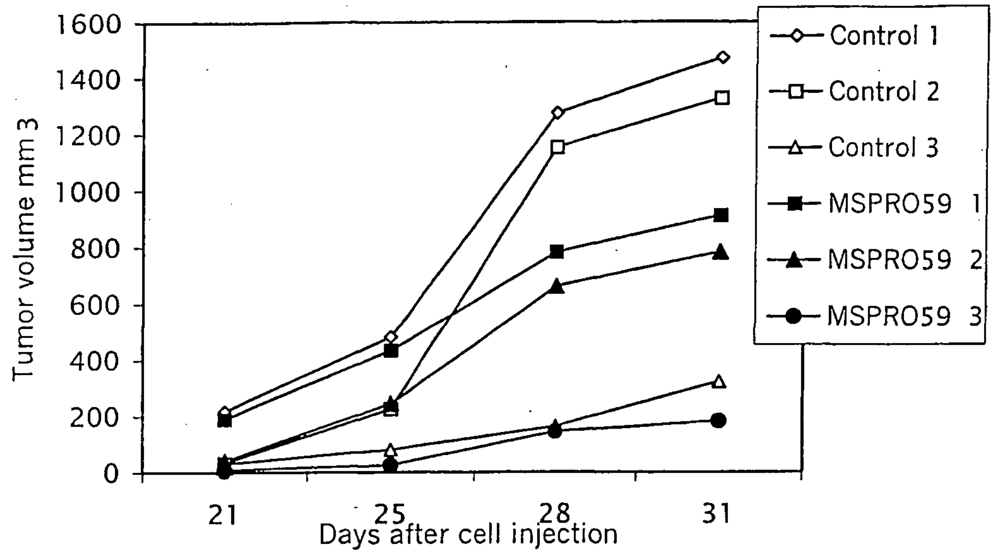


Figure 25B

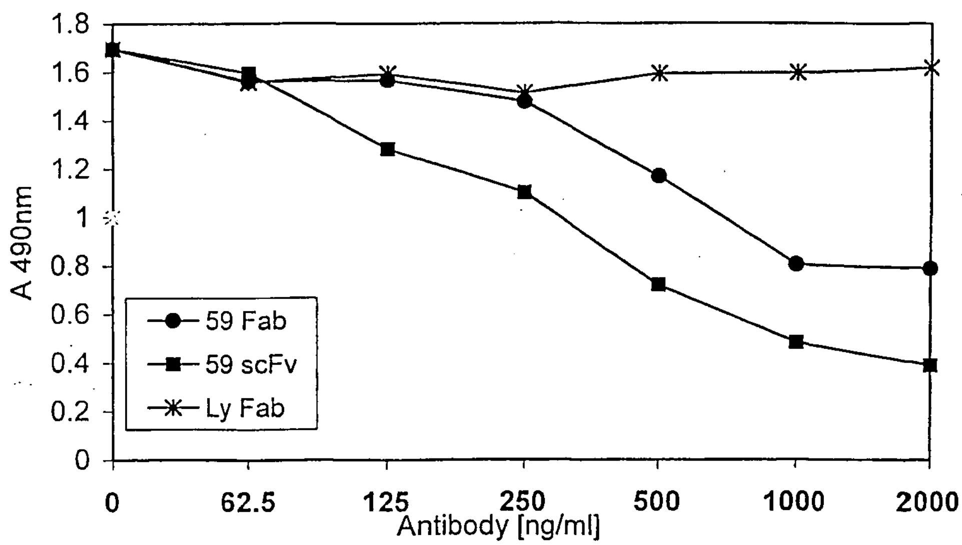
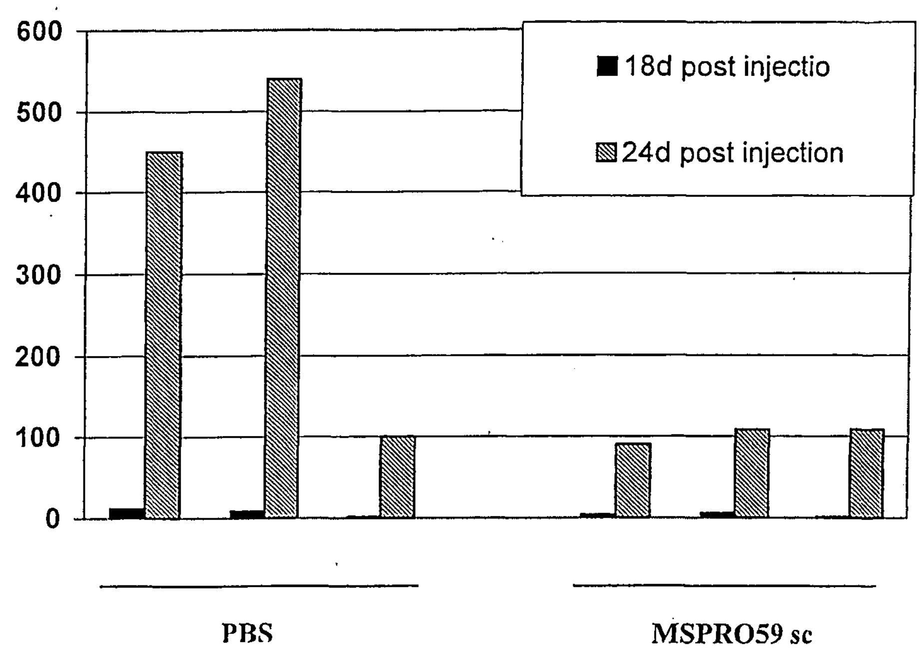


Figure 26



SUBSTITUTE SHEET (RULE 26)

Figure 27

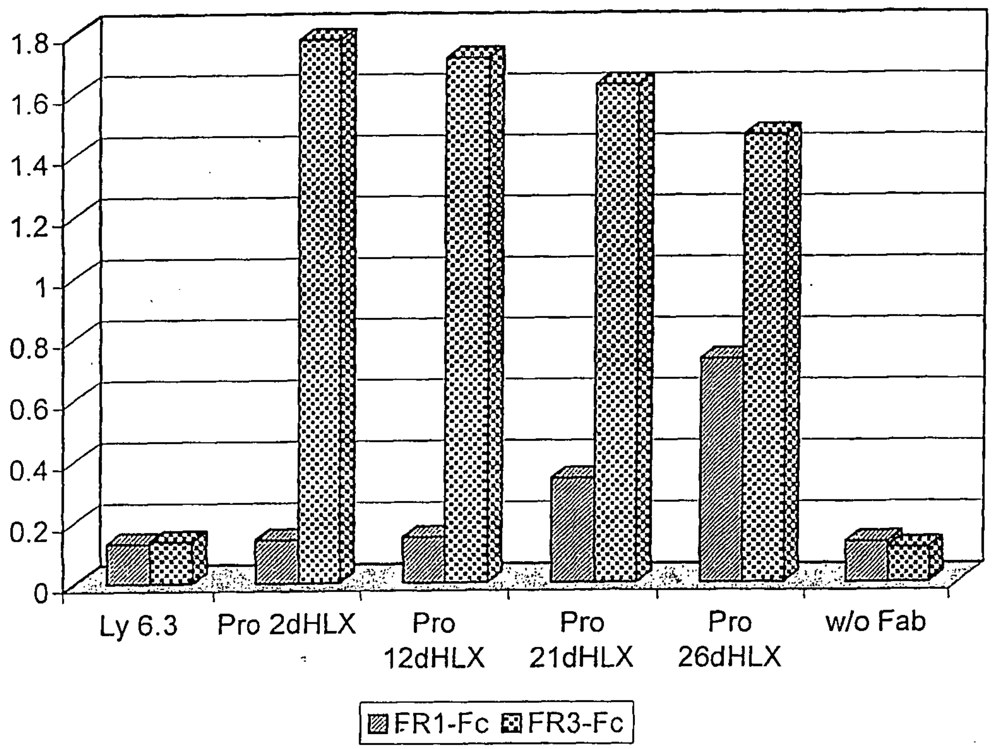

1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Ly 6.3
Pro 2dHLX
Pro 12dHLX
Pro 21dHLX
Pro 26dHLX
w/o Fab
FR1-Fc
FR3-Fc

Figure 28A

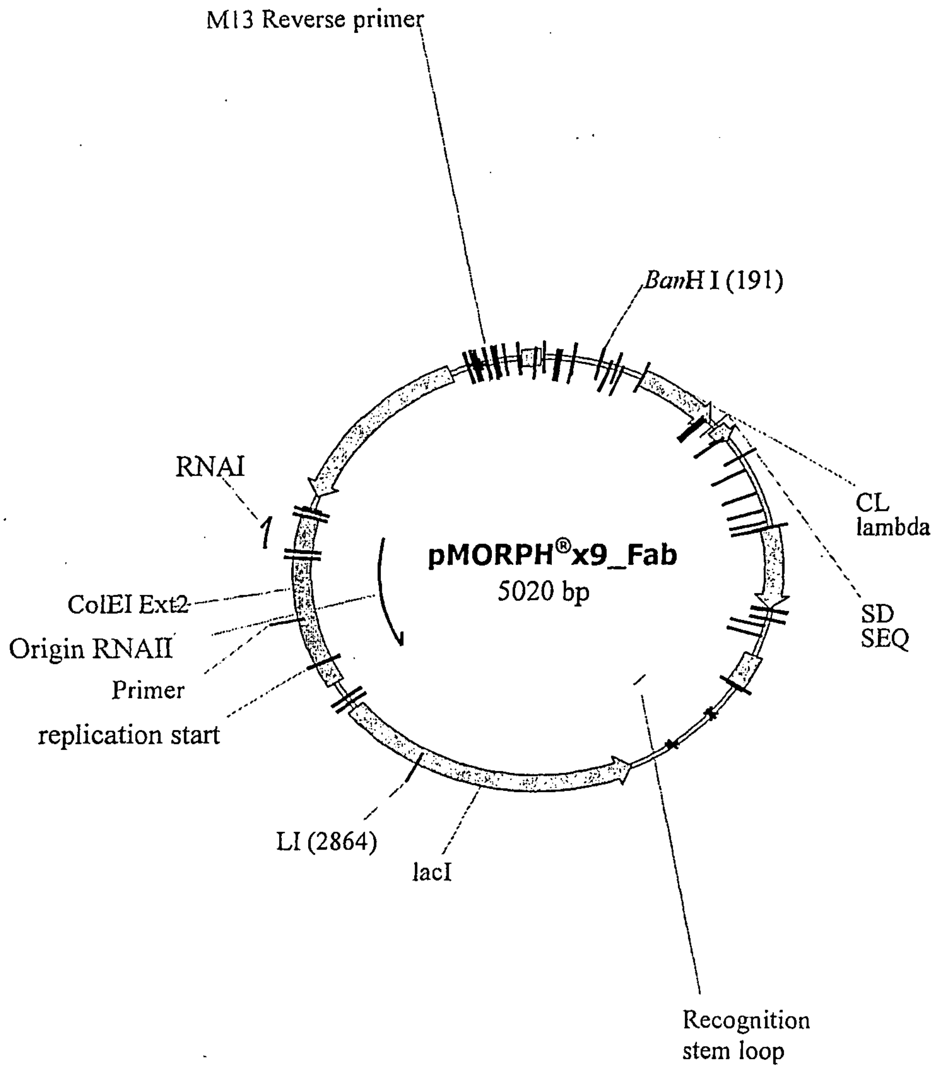

M13 Reverse primer
BamH I (191)
RNAI
1
pMORPH®x9_Fab
5020 bp
ColEI Ext2
Origin RNAII
Primer
replication start
CL
lambda
SD
SEQ
LI (2864)
lacI
Recognition
stem loop

Figure 28B

EcoRV SexAI

SEQ ID NO:52 1 ATCGTGCTGA CCCAGCCGCC TTCAGTGAGT GGCGCACCAG GTCAGCGTGT
TAGCACGACT GGGTCGGCGG AAGTCACTCA CCGCGTGGTC CAGTCGCACA

51 GACCATCTCG TGTAGCGGCA GCAGCAGCAA CATTGGCAGC AACTATGTGA
CTGGTAGAGC ACATCGCCGT CGTCGTCGTT GTAACCGTCG TTGATACACT

XmaI

KpnI SmaI

Acc65I AvaI

101 GCTGGTACCA GCAGTTGCCC GGGACGGCGC CGAAACTGCT GATTTATGAT
CGACCATGGT CGTCAACGGG CCCTGCCGCG GCTTTGACGA CTAAATACTA

Bsu36I BamHI

151 AACAACCAGC GTCCCTCAGG CGTGCCGGAT CGTTTTAGCG GATCCAAAAG
TTGTTGGTCG CAGGGAGTCC GCACGGCCTA GCAAAATCGC CTAGGTTTTC

BpuAI

BbsI

201 CGGCACCAGC GCGAGCCTTG CGATTACGGG CCTGCAAAGC GAAGACGAAG
GCCGTGGTCG CGCTCGGAAC GCTAATGCCC GGACGTTTCG CTTCTGCTTC

Bsu36I

251 CGGATTATTA TTGCCAGAGC TATGACATGC CTCAGGCTGT GTTTGGCGGC
GCCTAATAAT AACGGTCTCG ATACTGTACG GAGTCCGACA CAAACCGCCG

MscI DraIII

301 GGCACGAAGT TTAACCGTTC TTGGCCAGCC GAAAGCCGCA CCGAGTGTGA
CCGTGCTTCA AATTGGCAAG AACCGGTCGG CTTTCGGCGT GGCTCACACT

351 CGCTGTTTCC GCCGAGCAGC GAAGAATTGC AGGCGAACAA AGCGACCCTG
GCGACAAAGG CGGCTCGTCG CTTCTTAACG TCCGCTTGTT TCGCTGGGAC

401 GTGTGCCTGA TTAGCGACTT TTATCCGGGA GCCGTGACAG TGGCCTGGAA
CACACGGACT AATCGCTGAA AATAGGCCCT CGGCACTGTC ACCGGACCTT

451 GGCAGATAGC AGCCCCGTCA AGGCGGGAGT GGAGACCACC ACACCCTCCA
CCGTCTATCG TCGGGGCAGT TCCGCCCTCA CCTCTGGTGG TGTGGGAGGT

501 AACAAAGCAA CAACAAGTAC GCGGCCAGCA GCTATCTGAG CCTGACGCCT
TTGTTTCGTT GTTGTTCATG CGCCGGTCGT CGATAGACTC GGACTGCGGA

551 GAGCAGTGGA AGTCCCACAG AAGCTACAGC TGCCAGGTCA CGCATGAGGG
CTCGTCACCT TCAGGGTGTC TTCGATGTCG ACGGTCCAGT GCGTACTCCC

StuI SphI

601 GAGCACCGTG GAAAAAACCG TTGCGCCGAC TGAGGCCTGA TAAGCATGCG
CTCGTGGCAC CTTTTTTGGC AACGCGGCTG ACTCCGGACT ATTCGTACGC

651 TAGGAGAAAA TAAAATGAAA CAAAGCACTA TTGCACTGGC ACTCTTACCG
ATCCTCTTTT ATTTTACTTT GTTTCGTGAT AACGTGACCG TGAGAATGGC

MfeI

701 TTGCTCTTCA CCCCTGTTAC CAAAGCCCAG GTGCAATTGA AAGAAAGCGG
AACGAGAAGT GGGGACAATG GTTTCGGGTC CACGTTAACT TTCTTTCGCC

BspEI

751 CCCGGCCCTG GTGAAACCGA CCCAAACCCT GACCCTGACC TGTACCTTTT
GGGCCGGGAC CACTTTGGCT GGGTTTGGGA CTGGGACTGG ACATGGAAAA

BspEI

801 CCGGATTTAG CCTGTCCACG TCTGGCGTTG GCGTGGGCTG GATTCGCCAG
GGCCTAAATC GGACAGGTGC AGACCGCAAC CGCACCCGAC CTAAGCGGTC

XhoI

AvaI

851 CCGCCTGGGA AAGCCCTCGA GTGGCTGGCT CTGATTGATT GGGATGATGA
GGCGGACCCT TTCGGGAGCT CACCGACCGA GACTAACTAA CCCTACTACT

901 TAAGTATTAT AGCACCAGCC TGAAAACGCG TCTGACCATT AGCAAAGATA
ATTCATAATA TCGTGGTCGG ACTTTTGCGC AGACTGGTAA TCGTTTCTAT

BstBI

SfuI

NspV

951 CTTCGAAAAA TCAGGTGGTG CTGACTATGA CCAACATGGA CCCGGTGGAT
GAAGCTTTTT AGTCCACCAC GACTGATACT GGTTGTACCT GGGCCACCTA

BssHII

1001 ACGGCCACCT ATTATTGCGC GCGTTCTCCT CGTTATCGTG GTGCTTTTGA
TGCCGGTGGA TAATAACGCG CGCAAGAGGA GCAATAGCAC CACGAAAACT

BlpI

StyI CelII

1051 TTATTGGGGC CAAGGCACCC TGGTGACGGT TAGCTCAGCG TCGACCAAAG
AATAACCCCG GTTCCGTGGG ACCACTGCCA ATCGAGTCGC AGCTGGTTTC

1101 GTCCAAGCGT GTTTCCGCTG GCTCCGAGCA GCAAAAGCAC CAGCGGCGGC
CAGGTTCGCA CAAAGGCGAC CGAGGCTCGT CGTTTTCGTG GTCGCCGCCG

1151 ACGGCTGCCC TGGGCTGCCT GGTTAAAGAT TATTTCCCGG AACCAGTCAC
TGCCGACGGG ACCCGACGGA CCAATTTCTA ATAAAGGGCC TTGGTCAGTG

1201 CGTGAGCTGG AACAGCGGGG CGCTGACCAG CGGCGTGCAT ACCTTTCCGG
GCACTCGACC TTGTCGCCCC GCGACTGGTC GCCGCACGTA TGGAAAGGCC

1251 CGGTGCTGCA AAGCAGCGGC CTGTATAGCC TGAGCAGCGT TGTGACCGTG
GCCACGACGT TTCGTCGCCG GACATATCGG ACTCGTCGCA ACACTGGCAC

1301 CCGAGCAGCA GCTTAGGCAC TCAGACCTAT ATTTGCAACG TGAACCATAA
GGCTCGTCGT CGAATCCGTG AGTCTGGATA TAAACGTTGC ACTTGGTATT

EcoRI

1351 ACCGAGCAAC ACCAAAGTGG ATAAAAAAGT GGAACCGAAA AGCGAATTCG
TGGCTCGTTG TGGTTTCACC TATTTTTTCA CCTTGGCTTT TCGCTTAAGC

BssHII

1401 ACTATAAAGA TGACGATGAC AAAGGCGCGC CGTGGAGCCA CCCGCAGTTT
TGATATTTCT ACTGCTACTG TTTCCGCGCG GCACCTCGGT GGGCGTCAAA

HindIII

1451 GAAAAATGAT AAGCTTGACC TGTGAAGTGA AAAATGGCGC AGATTGTGCG
CTTTTTACTA TTCGAACTGG ACACTTCACT TTTTACCGCG TCTAACACGC

OGIII3 100.0%

1501 ACATTTTTTT TGTCTGCCGT TTAATTAAAG GGGGGGGGGG GCCGGCCTGG
TGTAAAAAAA ACAGACGGCA AATTAATTTC CCCCCCCCCC CGGCCGGACC

1551 GGGGGGGTGT ACATGAAATT GTAAACGTTA ATATTTTGTT AAAATTCGCG
CCCCCCCACA TGTACTTTAA CATTTGCAAT TATAAAACAA TTTTAAGCGC

1601 TTAAATTTTT GTTAAATCAG CTCATTTTTT AACCAATAGG CCGAAATCGG
AATTTAAAAA CAATTTAGTC GAGTAAAAAA TTGGTTATCC GGCTTTAGCC

1651 CAAAATCCCT TATAAATCAA AAGAATAGAC CGAGATAGGG TTGAGTGTTG
GTTTTAGGGA ATATTTAGTT TTCTTATCTG GCTCTATCCC AACTCACAAC

1701 TTCCAGTTTG GAACAAGAGT CCACTATTAA AGAACGTGGA CTCCAACGTC
AAGGTCAAAC CTTGTTCTCA GGTGATAATT TCTTGCACCT GAGGTTGCAG

1751 AAAGGGCGAA AAACCGTCTA TCAGGGCGAT GGCCCACTAC GAGAACCATC
TTTCCCGCTT TTTGGCAGAT AGTCCCGCTA CCGGGTGATG CTCTTGGTAG

1801 ACCCTAATCA AGTTTTTTGG GGTCGAGGTG CCGTAAAGCA CTAAATCGGA
TGGGATTAGT TCAAAAAACC CCAGCTCCAC GGCATTTCGT GATTTAGCCT

1851 ACCCTAAAGG GAGCCCCCGA TTTAGAGCTT GACGGGGAAA GCCGGCGAAC
TGGGATTTCC CTCGGGGGCT AAATCTCGAA CTGCCCCTTT CGGCCGCTTG

1901 GTGGCGAGAA AGGAAGGGAA GAAAGCGAAA GGAGCGGGCG CTAGGGCGCT
CACCGCTCTT TCCTTCCCTT CTTTCGCTTT CCTCGCCCGC GATCCCGCGA

1951 GGCAAGTGTA GCGGTCACGC TGCGCGTAAC CACCACACCC GCCGCGCTTA
CCGTTCACAT CGCCAGTGCG ACGCGCATTG GTGGTGTGGG CGGCGCGAAT

2001 ATGCGCCGCT ACAGGGCGCG TGCTAGACTA GTGTTTAAAC CGGACCGGGG
TACGCGGCGA TGTCCCGCGC ACGATCTGAT CACAAATTTG GCCTGGCCCC

2051 GGGGGCTTAA GTGGGCTGCA AAACAAAACG GCCTCCTGTC AGGAAGCCGC
CCCCCGAATT CACCCGACGT TTTGTTTTGC CGGAGGACAG TCCTTCGGCG

2101 TTTTATCGGG TAGCCTCACT GCCCGCTTTC CAGTCGGGAA ACCTGTCGTG
AAAATAGCCC ATCGGAGTGA CGGGCGAAAG GTCAGCCCTT TGGACAGCAC

2151 CCAGCTGCAT CAGTGAATCG GCCAACGCGC GGGGAGAGGC GGTTTGCGTA
GGTCGACGTA GTCACTTAGC CGGTTGCGCG CCCCTCTCCG CCAAACGCAT

2201 TTGGGAGCCA GGGTGGTTTT TCTTTTCACC AGTGAGACGG GCAACAGCTG
AACCCTCGGT CCCACCAAAA AGAAAAGTGG TCACTCTGCC CGTTGTCGAC

2251 ATTGCCCTTC ACCGCCTGGC CCTGAGAGAG TTGCAGCAAG CGGTCCACGC
TAACGGGAAG TGGCGGACCG GGACTCTCTC AACGTCGTTC GCCAGGTGCG

2301 TGGTTTGCCC CAGCAGGCGA AAATCCTGTT TGATGGTGGT CAGCGGCGGG
ACCAAACGGG GTCGTCCGCT TTTAGGACAA ACTACCACCA GTCGCCGCCC

2351 ATATAACATG AGCTGTCCTC GGTATCGTCG TATCCCACTA CCGAGATGTC
TATATTGTAC TCGACAGGAG CCATAGCAGC ATAGGGTGAT GGCTCTACAG

2401 CGCACCAACG CGCAGCCCGG ACTCGGTAAT GGCACGCATT GCGCCCAGCG
GCGTGGTTGC GCGTCGGGCC TGAGCCATTA CCGTGCGTAA CGCGGGTCGC

2451 CCATCTGATC GTTGGCAACC AGCATCGCAG TGGGAACGAT GCCCTCATTC
GGTAGACTAG CAACCGTTGG TCGTAGCGTC ACCCTTGCTA CGGGAGTAAG

2501 AGCATTTGCA TGGTTTGTTG AAAACCGGAC ATGGCACTCC AGTCGCCTTC
TCGTAAACGT ACCAAACAAC TTTTGGCCTG TACCGTGAGG TCAGCGGAAG

2551 CCGTTCCGCT ATCGGCTGAA TTTGATTGCG AGTGAGATAT TTATGCCAGC
GGCAAGGCGA TAGCCGACTT AAACTAACGC TCACTCTATA AATACGGTCG

2601 CAGCCAGACG CAGACGCGCC GAGACAGAAC TTAATGGGCC AGCTAACAGC
GTCGGTCTGC GTCTGCGCGG CTCTGTCTTG AATTACCCGG TCGATTGTCG

2651 GCGATTTGCT GGTGGCCCAA TGCGACCAGA TGCTCCACGC CCAGTCGCGT
CGCTAAACGA CCACCGGGTT ACGCTGGTCT ACGAGGTGCG GGTCAGCGCA

2701 ACCGTCCTCA TGGGAGAAAA TAATACTGTT GATGGGTGTC TGGTCAGAGA
TGGCAGGAGT ACCCTCTTTT ATTATGACAA CTACCCACAG ACCAGTCTCT

2751 CATCAAGAAA TAACGCCGGA ACATTAGTGC AGGCAGCTTC CACAGCAATA
GTAGTTCTTT ATTGCGGCCT TGTAATCACG TCCGTCGAAG GTGTCGTTAT

2801 GCATCCTGGT CATCCAGCGG ATAGTTAATA ATCAGCCCAC TGACACGTTG
CGTAGGACCA GTAGGTCGCC TATCAATTAT TAGTCGGGTG ACTGTGCAAC

ApaLI

2851 CGCGAGAAGA TTGTGCACCG CCGCTTTACA GGCTTCGACG CCGCTTCGTT
GCGCTCTTCT AACACGTGGC GGCGAAATGT CCGAAGCTGC GGCGAAGCAA

2901 CTACCATCGA CACGACCACG CTGGCACCCA GTTGATCGGC GCGAGATTTA

GATGGTAGCT GTGCTGGTGC GACCGTGGGT CAACTAGCCG CGCTCTAAAT

2951 ATCGCCGCGA CAATTTGCGA CGGCGCGTGC AGGGCCAGAC TGGAGGTGGC
TAGCGGCGCT GTTAAACGCT GCCGCGCACG TCCCGGTCTG ACCTCCACCG

3001 AACGCCAATC AGCAACGACT GTTTGCCCGC CAGTTGTTGT GCCACGCGGT
TTGCGGTTAG TCGTTGCTGA CAAACGGGCG GTCAACAACA CGGTGCGCCA

3051 TAGGAATGTA ATTCAGCTCC GCCATCGCCG CTTCCACTTT TTCCCGCGTT
ATCCTTACAT TAAGTCGAGG CGGTAGCGGC GAAGGTGAAA AAGGGCGCAA

3101 TTCGCAGAAA CGTGGCTGGC CTGGTTCACC ACGCGGGAAA CGGTCTGATA
AAGCGTCTTT GCACCGACCG GACCAAGTGG TGCGCCCTTT GCCAGACTAT

3151 AGAGACACCG GCATACTCTG CGACATCGTA TAACGTTACT GGTTTCACAT
TCTCTGTGGC CGTATGAGAC GCTGTAGCAT ATTGCAATGA CCAAAGTGTA

3201 TCACCACCCT GAATTGACTC TCTTCCGGGC GCTATCATGC CATACCGCGA
AGTGGTGGGA CTTAACTGAG AGAAGGCCCG CGATAGTACG GTATGGCGCT

3251 AAGGTTTTGC GCCATTCGAT GCTAGCCATG TGAGCAAAAG GCCAGCAAAA
TTCCAAAACG CGGTAAGCTA CGATCGGTAC ACTCGTTTTC CGGTCGTTTT

3301 GGCCAGGAAC CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC
CCGGTCCTTG GCATTTTTCC GGCGCAACGA CCGCAAAAAG GTATCCGAGG

3351 GCCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA GAGGTGGCGA
CGGGGGGACT GCTCGTAGTG TTTTTAGCTG CGAGTTCAGT CTCCACCGCT

3401 AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCCCTG GAAGCTCCCT
TTGGGCTGTC CTGATATTTC TATGGTCCGC AAAGGGGGAC CTTCGAGGGA

3451 CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT
GCACGCGAGA GGACAAGGCT GGGACGGCGA ATGGCCTATG GACAGGCGGA

3501 TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT
AAGAGGGAAG CCCTTCGCAC CGCGAAAGAG TATCGAGTGC GACATCCATA

ApaLI

3551 CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC
GAGTCAAGCC ACATCCAGCA AGCGAGGTTC GACCCGACAC ACGTGCTTGG

3601 CCCCGTTCAG CCCGACCGCT GCGCCTTATC CGGTAACTAT CGTCTTGAGT
GGGGCAAGTC GGGCTGGCGA CGCGGAATAG GCCATTGATA GCAGAACTCA

3651 CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC
GGTTGGGCCA TTCTGTGCTG AATAGCGGTG ACCGTCGTCG GTGACCATTG

3701 AGGATTAGCA GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG
TCCTAATCGT CTCGCTCCAT ACATCCGCCA CGATGTCTCA AGAACTTCAC

3751 GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC
CACCGGATTG ATGCCGATGT GATCTTCTTG TCATAAACCA TAGACGCGAG

3801 TGCTGTAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC
ACGACATCGG TCAATGGAAG CCTTTTTCTC AACCATCGAG AACTAGGCCG

3851 AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT
TTTGTTTGGT GGCGACCATC GCCACCAAAA AAACAAACGT TCGTCGTCTA

3901 TACGCGCAGA AAAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTTCTACGG
ATGCGCGTCT TTTTTTCCTA GAGTTCTTCT AGGAAACTAG AAAAGATGCC

3951 GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT TTTGGTCAGA
CCAGACTGCG AGTCACCTTG CTTTTGAGTG CAATTCCCTA AAACCAGTCT

4001 TCTAGCACCA GGCGTTTAAG GGCACCAATA ACTGCCTTAA AAAAATTACG
AGATCGTGGT CCGCAAATTC CCGTGGTTAT TGACGGAATT TTTTTAATGC

4051 CCCCGCCCTG CCACTCATCG CAGTACTGTT GTAATTCATT AAGCATTCTG
GGGGCGGGAC GGTGAGTAGC GTCATGACAA CATTAAGTAA TTCGTAAGAC

4101 CCGACATGGA AGCCATCACA AACGGCATGA TGAACCTGAA TCGCCAGCGG
GGCTGTACCT TCGGTAGTGT TTGCCGTACT ACTTGGACTT AGCGGTCGCC

4151 CATCAGCACC TTGTCGCCTT GCGTATAATA TTTGCCCATA GTGAAAACGG
GTAGTCGTGG AACAGCGGAA CGCATATTAT AAACGGGTAT CACTTTTGCC

4201 GGGCGAAGAA GTTGTCCATA TTGGCTACGT TTAAATCAAA ACTGGTGAAA
CCCGCTTCTT CAACAGGTAT AACCGATGCA AATTTAGTTT TGACCACTTT

4251 CTCACCCAGG GATTGGCTGA GACGAAAAAC ATATTCTCAA TAAACCCTTT
GAGTGGGTCC CTAACCGACT CTGCTTTTTG TATAAGAGTT ATTTGGGAAA

4301 AGGGAAATAG GCCAGGTTTT CACCGTAACA CGCCACATCT TGCGAATATA
TCCCTTTATC CGGTCCAAAA GTGGCATTGT GCGGTGTAGA ACGCTTATAT

4351 TGTGTAGAAA CTGCCGGAAA TCGTCGTGGT ATTCACTCCA GAGCGATGAA
ACACATCTTT GACGGCCTTT AGCAGCACCA TAAGTGAGGT CTCGCTACTT

4401 AACGTTTCAG TTTGCTCATG GAAAACGGTG TAACAAGGGT GAACACTATC
TTGCAAAGTC AAACGAGTAC CTTTTGCCAC ATTGTTCCCA CTTGTGATAG

4451 CCATATCACC AGCTCACCGT CTTTCATTGC CATACGGAAC TCCGGGTGAG
GGTATAGTGG TCGAGTGGCA GAAAGTAACG GTATGCCTTG AGGCCCACTC

4501 CATTCATCAG GCGGGCAAGA ATGTGAATAA AGGCCGGATA AAACTTGTGC
GTAAGTAGTC CGCCCGTTCT TACACTTATT TCCGGCCTAT TTTGAACACG

4551 TTATTTTTCT TTACGGTCTT TAAAAAGGCC GTAATATCCA GCTGAACGGT
AATAAAAAGA AATGCCAGAA ATTTTTCCGG CATTATAGGT CGACTTGCCA

4601 CTGGTTATAG GTACATTGAG CAACTGACTG AAATGCCTCA AAATGTTCTT
GACCAATATC CATGTAACTC GTTGACTGAC TTTACGGAGT TTTACAAGAA

4651 TACGATGCCA TTGGGATATA TCAACGGTGG TATATCCAGT GATTTTTTTC
ATGCTACGGT AACCCTATAT AGTTGCCACC ATATAGGTCA CTAAAAAAAG

4701 TCCATTTTAG CTTCCTTAGC TCCTGAAAAT CTCGATAACT CAAAAAATAC
AGGTAAAATC GAAGGAATCG AGGACTTTTA GAGCTATTGA GTTTTTTATG

4751 GCCCGGTAGT GATCTTATTT CATTATGGTG AAAGTTGGAA CCTCACCCGA
CGGGCCATCA CTAGAATAAA GTAATACCAC TTTCAACCTT GGAGTGGGCT

4801 CGTCTAATGT GAGTTAGCTC ACTCATTAGG CACCCCAGGC TTTACACTTT
GCAGATTACA CTCAATCGAG TGAGTAATCC GTGGGGTCCG AAATGTGAAA

4851 ATGCTTCCGG CTCGTATGTT GTGTGGAATT GTGAGCGGAT AACAATTTCA
TACGAAGGCC GAGCATACAA CACACCTTAA CACTCGCCTA TTGTTAAAGT

M13 Reverse primer 100.0% XbaI

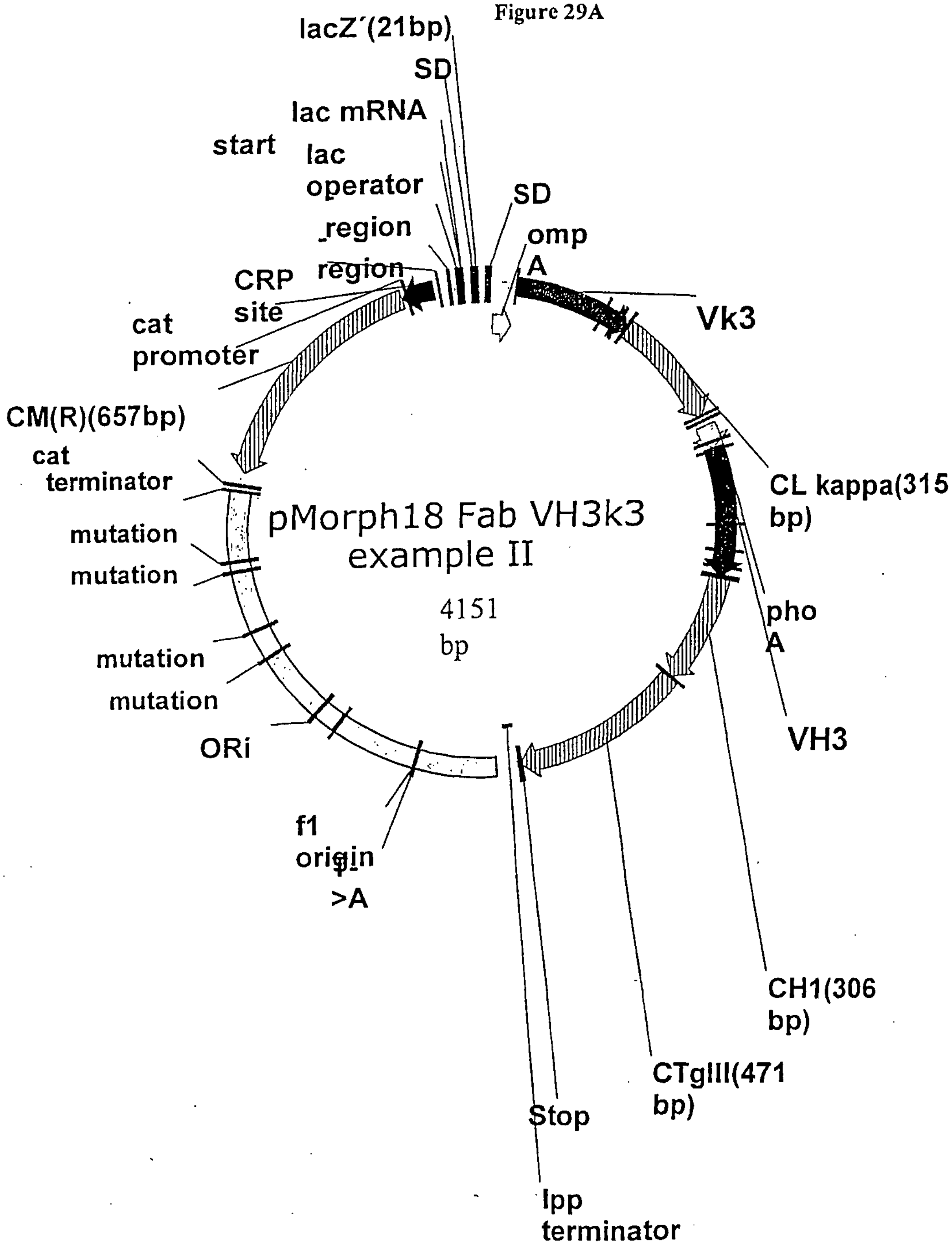
4901 CACAGGAAAC AGCTATGACC ATGATTACGA ATTTCTAGAT AACGAGGGCA
GTGTCCTTTG TCGATACTGG TACTAATGCT TAAAGATCTA TTGCTCCCGT

4951 AAAAATGAAA AAGACAGCTA TCGCGATTGC AGTGGCACTG GCTGGTTTCG
TTTTTACTTT TTCTGTCGAT AGCGCTAACG TCACCGTGAC CGACCAAAGC

EcoRV

5001 CTACCGTAGC GCAGGCCGAT
GATGGCATCG CGTCCGGCTA

Figure 29A



SUBSTITUTE SHEET (RULE 26)

Figure 29B

```
         lacZ'   SD          ompA
         XbaI
                                        M K K T A I A I A V·
SEQ ID NO:53 TCTAGATAAC GAGGGCAAAA AATGAAAAAG ACAGCTATCG CGATTGCAGT
             AGATCTATTG CTCCCGTTTT TTACTTTTTC TGTCGATAGC GCTAACGTCA
                             Vk3
             ompA
                           EcoRV
         A L A G F A T V A Q A D I V L T Q·
         GGCACTGGCT GGTTTCGCTA CCGTAGCGCA GGCCGATATC GTGCTGACCC
         CCGTGACCGA CCAAAGCGAT GGCATCGCGT CCGGCTATAG CACGACTGGG
                   Vk3
         ·S P A T L S L S P G E R A T L S
   101   AGAGCCCGGC GACCCTGAGC CTGTCTCCGG GCGAACGTGC GACCCTGAGC
         TCTCGGGCCG CTGGGACTCG GACAGAGGCC CGCTTGCACG CTGGGACTCG
                   Vk3
         C R A S Q S V S S S Y L A W Y Q Q·
   151   TGCAGAGCGA GCCAGAGCGT GAGCAGCAGC TATCTGGCGT GGTACCAGCA
         ACGTCTCGCT CGGTCTCGCA CTCGTCGTCG ATAGACCGCA CCATGGTCGT
                   Vk3
         ·K P G Q A P R L L I Y G A S S R A·
   201   GAAACCAGGT CAAGCACCGC GTCTATTAAT TTATGGCGCG AGCAGCCGTG
         CTTTGGTCCA GTTCGTGGCG CAGATAATTA AATACCGCGC TCGTCGGCAC
                   Vk3
         ·T G V P A R F S G S G S G T D F
   251   CAACTGGGGT CCCGGCGCGT TTTAGCGGCT CTGGATCCGG CACGGATTTT
         GTTGACCCCA GGGCCGCGCA AAATCGCCGA GACCTAGGCC GTGCCTAAAA
                   Vk3
                      BbsI
         T L T I S S L E P E D F A V Y Y C·
   301   ACCCTGACCA TTAGCAGCCT GGAACCTGAA GACTTTGCGG TGTATTATTG
         TGGGACTGGT AATCGTCGGA CCTTGGACTT CTGAAACGCC ACATAATAAC
                   Vk3
                        MscI
         ·Q Q H Y T T P P T F G Q G T K V E·
   351   CCAGCAGCAT TATACCACCC CGCCGACCTT TGGCCAGGGT ACGAAAGTTG
         GGTCGTCGTA ATATGGTGGG GCGGCTGGAA ACCGGTCCCA TGCTTTCAAC
                      CL kappa
          Vk3
           BsiWI
         ·I K R T V A A P S V F I F P P S
   401   AAATTAAACG TACGGTGGCT GCTCCGAGCG TGTTTATTTT TCCGCCGAGC
         TTTAATTTGC ATGCCACCGA CGAGGCTCGC ACAAATAAAA AGGCGGCTCG
```

```
                CL kappa
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
           D E Q L K S G T A S V V C L L N N·
451        GATGAACAAC TGAAAAGCGG CACGGCGAGC GTGGTGTGCC TGCTGAACAA
           CTACTTGTTG ACTTTTCGCC GTGCCGCTCG CACCACACGG ACGACTTGTT
                CL kappa
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
           ·F Y P R E A K V Q W K V D N A L Q·
501        CTTTTATCCG CGTGAAGCGA AAGTTCAGTG GAAAGTAGAC AACGCGCTGC
           GAAAATAGGC GCACTTCGCT TTCAAGTCAC CTTTCATCTG TTGCGCGACG
                CL kappa
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
           · S G N S Q E S V T E Q D S K D S
551        AAAGCGGCAA CAGCCAGGAA AGCGTGACCG AACAGGATAG CAAAGATAGC
           TTTCGCCGTT GTCGGTCCTT TCGCACTGGC TTGTCCTATC GTTTCTATCG
                CL kappa
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
           T Y S L S S T L T L S K A D Y E K·
601        ACCTATTCTC TGAGCAGCAC CCTGACCCTG AGCAAAGCGG ATTATGAAAA
           TGGATAAGAG ACTCGTCGTG GGACTGGGAC TCGTTTCGCC TAATACTTTT
                CL kappa
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
           ·H K V Y A C E V T H Q G L S S P V·
651        ACATAAAGTG TATGCGTGCG AAGTGACCCA TCAAGGTCTG AGCAGCCCGG
           TGTATTTCAC ATACGCACGC TTCACTGGGT AGTTCCAGAC TCGTCGGGCC
            CL kappa
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~
                      StuI    SphI
                      ~~~~~~  ~~~~~~
           · T K S F N R G E A
701        TGACTAAATC TTTTAATCGT GGCGAGGCCT GATAAGCATG CGTAGGAGAA
           ACTGATTTAG AAAATTAGCA CCGCTCCGGA CTATTCGTAC GCATCCTCTT
                      phoA
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                               SapI
                               ~~~~~
              M K Q S T I A L A L L P L L F·
751        AATAAAATGA AACAAAGCAC TATTGCACTG GCACTCTTAC CGTTGCTCTT
           TTATTTTACT TTGTTTCGTG ATAACGTGAC CGTGAGAATG GCAACGAGAA
                          VH3
                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

             phoA
           ~~~~~~~~~~~~~~~~~
           SapI         MfeI
           ~            ~~~~~
           ·T P V T K A Q V Q L V E S G G G L·
801        CACCCCTGTT ACCAAAGCCG AAGTGCAATT GGTGGAAAGC GGCGGCGGCC
           GTGGGGACAA TGGTTTCGGC TTCACGTTAA CCACCTTTCG CCGCCGCCGG
                  VH3
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
           · V Q P G G S L R L S C A A S G F
851        TGGTGCAACC GGGCGGCAGC CTGCGTCTGA GCTGCGCGGC CTCCGGATTT
           ACCACGTTGG CCCGCCGTCG GACGCAGACT CGACGCGCCG GAGGCCTAAA
                  VH3
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
           T F S S Y A M S W V R Q A P G K G·
901        ACCTTTAGCA GCTATGCGAT GAGCTGGGTG CGCCAAGCCC CTGGGAAGGG
           TGGAAATCGT CGATACGCTA CTCGACCCAC GCGGTTCGGG GACCCTTCCC
                  VH3
           ~~~~~~~~~~~~~~~~~~            ~~~~~~~~~~~~~~~~~~
```

```
     ·L E W  V S A I  S G S  G G S  T Y Y A·
951  TCTCGAGTGG GTGAGCGCGA TTAGCGGTAG CGGCGGCAGC ACCTATTATG
     AGAGCTCACC CACTCGCGCT AATCGCCATC GCCGCCGTCG TGGATAATAC
                VH3

                       PmlI

     ·D S V  K G R  F T I S  R D N  S K N
1001 CGGATAGCGT GAAAGGCCGT TTTACCATTT CACGTGATAA TTCGAAAAAC
     GCCTATCGCA CTTTCCGGCA AAATGGTAAA GTGCACTATT AAGCTTTTTG
                VH3

     T L Y L  Q M N  S L R  A E D T  A V Y·
1051 ACCCTGTATC TGCAAATGAA CAGCCTGCGT GCGGAAGATA CGGCCGTGTA
     TGGGACATAG ACGTTTACTT GTCGGACGCA CGCCTTCTAT GCCGGCACAT
                VH3

      BssHII

     ·Y C A  R W G G  D G F  Y A M  D Y W G·
1101 TTATTGCGCG CGTTGGGGCG GCGATGGCTT TTATGCGATG GATTATTGGG
     AATAACGCGC GCAACCCCGC CGCTACCGAA AATACGCTAC CTAATAACCC
                          CH1

          VH3

                       SalI

      StyI        BlpI

     ·Q G T  L V T  V S S A  S T K  G P S
1151 GCCAAGGCAC CCTGGTGACG GTTAGCTCAG CGTCGACCAA AGGTCCAAGC
     CGGTTCCGTG GGACCACTGC CAATCGAGTC GCAGCTGGTT TCCAGGTTCG
                CH1

     V F P L  A P S  S K S  T S G G  T A A·
1201 GTGTTTCCGC TGGCTCCGAG CAGCAAAAGC ACCAGCGGCG GCACGGCTGC
     CACAAAGGCG ACCGAGGCTC GTCGTTTTCG TGGTCGCCGC CGTGCCGACG
                CH1

     ·L G C  L V K D  Y F P  E P V  T V S W·
1251 CCTGGGCTGC CTGGTTAAAG ATTATTTCCC GGAACCAGTC ACCGTGAGCT
     GGACCCGACG GACCAATTTC TAATAAAGGG CCTTGGTCAG TGGCACTCGA
                CH1

     ·N S G  A L T  S G V H  T F P  A V L
1301 GGAACAGCGG GGCGCTGACC AGCGGCGTGC ATACCTTTCC GGCGGTGCTG
     CCTTGTCGCC CCGCGACTGG TCGCCGCACG TATGGAAAGG CCGCCACGAC
                CH1

     Q S S G  L Y S  L S S  V V T V  P S S·
1351 CAAAGCAGCG GCCTGTATAG CCTGAGCAGC GTTGTGACCG TGCCGAGCAG
     GTTTCGTCGC CGGACATATC GGACTCGTCG CAACACTGGC ACGGCTCGTC
                CH1

     ·S L G  T Q T Y  I C N  V N H  K P S N·
1401 CAGCTTAGGC ACTCAGACCT ATATTTGCAA CGTGAACCAT AAACCGAGCA
     GTCGAATCCG TGAGTCTGGA TATAAACGTT GCACTTGGTA TTTGGCTCGT
             CH1            CTgIII
```

```
                              EcoRI
                              ~~~~~
       ·T K V  D K K  V E P K  S E F  G G G
1451   ACACCAAAGT GGATAAAAAA GTGGAACCGA AAAGCGAATT CGGGGGAGGG
       TGTGGTTTCA CCTATTTTTT CACCTTGGCT TTTCGCTTAA GCCCCCTCCC
                  CTgIII
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       S G S G  D F D  Y E K  M A N A  N K G·
1501   AGCGGGAGCG GTGATTTTGA TTATGAAAAG ATGGCAAACG CTAATAAGGG
       TCGCCCTCGC CACTAAAACT AATACTTTTC TACCGTTTGC GATTATTCCC
                  CTgIII
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       ·A M T  E N A D  E N A  L Q S  D A K G·
1551   GGCTATGACC GAAAATGCCG ATGAAAACGC GCTACAGTCT GACGCTAAAG
       CCGATACTGG CTTTTACGGC TACTTTTGCG CGATGTCAGA CTGCGATTTC
                  CTgIII
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       ·K L D  S V A  T D Y G  A A I  D G F
1601   GCAAACTTGA TTCTGTCGCT ACTGATTACG GTGCTGCTAT CGATGGTTTC
       CGTTTGAACT AAGACAGCGA TGACTAATGC CACGACGATA GCTACCAAAG
                  CTgIII
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       I G D V  S G L  A N G  N G A T  G D F·
1651   ATTGGTGACG TTTCCGGCCT TGCTAATGGT AATGGTGCTA CTGGTGATTT
       TAACCACTGC AAAGGCCGGA ACGATTACCA TTACCACGAT GACCACTAAA
                  CTgIII
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       ·A G S  N S Q M  A Q V  G D G  D N S P·
1701   TGCTGGCTCT AATTCCCAAA TGGCTCAAGT CGGTGACGGT GATAATTCAC
       ACGACCGAGA TTAAGGGTTT ACCGAGTTCA GCCACTGCCA CTATTAAGTG
                  CTgIII
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       ·L M N  N F R  Q Y L P  S L P  Q S V
1751   CTTTAATGAA TAATTTCCGT CAATATTTAC CTTCCCTCCC TCAATCGGTT
       GAAATTACTT ATTAAAGGCA GTTATAAATG GAAGGGAGGG AGTTAGCCAA
                  CTgIII
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       E C R P  F V F  G A G  K P Y E  F S I·
1801   GAATGTCGCC CTTTTGTCTT TGGCGCTGGT AAACCATATG AATTTTCTAT
       CTTACAGCGG GAAAACAGAA ACCGCGACCA TTTGGTATAC TTAAAAGATA
                  CTgIII
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       ·D C D  K I N L  F R G  V F A  F L L Y·
1851   TGATTGTGAC AAAATAAACT TATTCCGTGG TGTCTTTGCG TTTCTTTTAT
       ACTAACACTG TTTTATTTGA ATAAGGCACC ACAGAAACGC AAAGAAAATA
                  CTgIII
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       ·V A T  F M Y  V F S T  F A N  I L R
1901   ATGTTGCCAC CTTTATGTAT GTATTTTCTA CGTTTGCTAA CATACTGCGT
       TACAACGGTG GAAATACATA CATAAAAGAT GCAAACGATT GTATGACGCA
        CTgIII
       ~~~~~~~~~~~~
             Stop        lpp terminator
             ~~~      ~~~~~~~~~~~~~~~~~~~~~~~~~~
               HindIII
               ~~~~~~
       N K E S
1951   AATAAGGAGT CTTGATAAGC TTGACCTGTG AAGTGAAAAA TGGCGCAGAT
       TTATTCCTCA GAACTATTCG AACTGGACAC TTCACTTTTT ACCGCGTCTA
       lpp terminator
```

2001 TGTGCGACAT TTTTTTTGTC TGCCGTTTAA TGAAATTGTA AACGTTAATA
ACACGCTGTA AAAAAAACAG ACGGCAAATT ACTTTAACAT TTGCAATTAT

f1 origin
2051 TTTTGTTAAA ATTCGCGTTA AATTTTTGTT AAATCAGCTC ATTTTTTAAC
AAAACAATTT TAAGCGCAAT TTAAAAACAA TTTAGTCGAG TAAAAAATTG

f1 origin
2101 CAATAGGCCG AAATCGGCAA AATCCCTTAT AAATCAAAAG AATAGACCGA
GTTATCCGGC TTTAGCCGTT TTAGGGAATA TTTAGTTTTC TTATCTGGCT

f1 origin
2151 GATAGGGTTG AGTGTTGTTC CAGTTTGGAA CAAGAGTCCA CTATTAAAGA
CTATCCCAAC TCACAACAAG GTCAAACCTT GTTCTCAGGT GATAATTTCT

f1 origin
2201 ACGTGGACTC CAACGTCAAA GGGCGAAAAA CCGTCTATCA GGGCGATGGC
TGCACCTGAG GTTGCAGTTT CCCGCTTTTT GGCAGATAGT CCCGCTACCG

f1 origin
T->A
2251 CCACTACGAG AACCATCACC CTAATCAAGT TTTTTGGGGT CGAGGTGCCG
GGTGATGCTC TTGGTAGTGG GATTAGTTCA AAAAACCCCA GCTCCACGGC

f1 origin
2301 TAAAGCACTA AATCGGAACC CTAAAGGGAG CCCCCGATTT AGAGCTTGAC
ATTTCGTGAT TTAGCCTTGG GATTTCCCTC GGGGGCTAAA TCTCGAACTG

f1 origin
2351 GGGGAAAGCC GGCGAACGTG GCGAGAAAGG AAGGGAAGAA AGCGAAAGGA
CCCCTTTCGG CCGCTTGCAC CGCTCTTTCC TTCCCTTCTT TCGCTTTCCT

f1 origin
2401 GCGGGCGCTA GGGCGCTGGC AAGTGTAGCG GTCACGCTGC GCGTAACCAC
CGCCCGCGAT CCCGCGACCG TTCACATCGC CAGTGCGACG CGCATTGGTG

f1 origin
NheI
2451 CACACCCGCC GCGCTTAATG CGCCGCTACA GGGCGCGTGC TAGCCATGTG
GTGTGGGCGG CGCGAATTAC GCGGCGATGT CCCGCGCACG ATCGGTACAC

f1 origin ColEI
2501 AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC GCGTTGCTGG
TCGTTTTCCG GTCGTTTTCC GGTCCTTGGC ATTTTTCCGG CGCAACGACC

ColEI
ORI
2551 CGTTTTTCCA TAGGCTCCGC CCCCCTGACG AGCATCACAA AAATCGACGC
GCAAAAAGGT ATCCGAGGCG GGGGGACTGC TCGTAGTGTT TTTAGCTGCG

ColEI
2601 TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT ACCAGGCGTT
AGTTCAGTCT CCACCGCTTT GGGCTGTCCT GATATTTCTA TGGTCCGCAA

ColEI
2651 TCCCCCTGGA AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA

AGGGGGACCT TCGAGGGAGC ACGCGAGAGG ACAAGGCTGG GACGGCGAAT

ColEI

mutation

2701 CCGGATACCT GTCCGCCTTT CTCCCTTCGG GAAGCGTGGC GCTTTCTCAT
GGCCTATGGA CAGGCGGAAA GAGGGAAGCC CTTCGCACCG CGAAAGAGTA

ColEI

mutation

2751 AGCTCACGCT GTAGGTATCT CAGTTCGGTG TAGGTCGTTC GCTCCAAGCT
TCGAGTGCGA CATCCATAGA GTCAAGCCAC ATCCAGCAAG CGAGGTTCGA

ColEI

mutation

2801 GGGCTGTGTG CACGAACCCC CCGTTCAGTC CGACCGCTGC GCCTTATCCG
CCCGACACAC GTGCTTGGGG GGCAAGTCAG GCTGGCGACG CGGAATAGGC

ColEI

2851 GTAACTATCG TCTTGAGTCC AACCCGGTAA GACACGACTT ATCGCCACTG
CATTGATAGC AGAACTCAGG TTGGGCCATT CTGTGCTGAA TAGCGGTGAC

ColEI

2901 GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG TAGGCGGTGC
CGTCGTCGGT GACCATTGTC CTAATCGTCT CGCTCCATAC ATCCGCCACG

ColEI

mutation

2951 TACAGAGTTC TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGAACAG
ATGTCTCAAG AACTTCACCA CCGGATTGAT GCCGATGTGA TCTTCTTGTC

ColEI

mutation

3001 TATTTGGTAT CTGCGCTCTG CTGTAGCCAG TTACCTTCGG AAAAAGAGTT
ATAAACCATA GACGCGAGAC GACATCGGTC AATGGAAGCC TTTTTCTCAA

ColEI

3051 GGTAGCTCTT GATCCGGCAA ACAAACCACC GCTGGTAGCG GTGGTTTTTT
CCATCGAGAA CTAGGCCGTT TGTTTGGTGG CGACCATCGC CACCAAAAAA

ColEI

3101 TGTTTGCAAG CAGCAGATTA CGCGCAGAAA AAAAGGATCT CAAGAAGATC
ACAAACGTTC GTCGTCTAAT GCGCGTCTTT TTTTCCTAGA GTTCTTCTAG

ColEI

3151 CTTTGATCTT TTCTACGGGG TCTGACGCTC AGTGGAACGA AAACTCACGT
GAAACTAGAA AAGATGCCCC AGACTGCGAG TCACCTTGCT TTTGAGTGCA

ColEI

cat terminator

BglII

3201 TAAGGGATTT TGGTCAGATC TAGCACCAGG CGTTTAAGGG CACCAATAAC
ATTCCCTAAA ACCAGTCTAG ATCGTGGTCC GCAAATTCCC GTGGTTATTG

ColEI
cat terminator

3251 TGCCTTAAAA AAATTACGCC CCGCCCTGCC ACTCATCGCA GTACTGTTGT
ACGGAATTTT TTTAATGCGG GGCGGGACGG TGAGTAGCGT CATGACAACA

CM(R)
3301 AATTCATTAA GCATTCTGCC GACATGGAAG CCATCACAAA CGGCATGATG
TTAAGTAATT CGTAAGACGG CTGTACCTTC GGTAGTGTTT GCCGTACTAC

CM(R)
3351 AACCTGAATC GCCAGCGGCA TCAGCACCTT GTCGCCTTGC GTATAATATT
TTGGACTTAG CGGTCGCCGT AGTCGTGGAA CAGCGGAACG CATATTATAA

CM(R)
3401 TGCCCATAGT GAAAACGGGG GCGAAGAAGT TGTCCATATT GGCTACGTTT
ACGGGTATCA CTTTTGCCCC CGCTTCTTCA ACAGGTATAA CCGATGCAAA

CM(R)
3451 AAATCAAAAC TGGTGAAACT CACCCAGGGA TTGGCTGAGA CGAAAAACAT
TTTAGTTTTG ACCACTTTGA GTGGGTCCCT AACCGACTCT GCTTTTTGTA

CM(R)
3501 ATTCTCAATA AACCCTTTAG GGAAATAGGC CAGGTTTTCA CCGTAACACG
TAAGAGTTAT TTGGGAAATC CCTTTATCCG GTCCAAAAGT GGCATTGTGC

CM(R)
3551 CCACATCTTG CGAATATATG TGTAGAAACT GCCGGAAATC GTCGTGGTAT
GGTGTAGAAC GCTTATATAC ACATCTTTGA CGGCCTTTAG CAGCACCATA

CM(R)
3601 TCACTCCAGA GCGATGAAAA CGTTTCAGTT TGCTCATGGA AAACGGTGTA
AGTGAGGTCT CGCTACTTTT GCAAAGTCAA ACGAGTACCT TTTGCCACAT

CM(R)
3651 ACAAGGGTGA ACACTATCCC ATATCACCAG CTCACCGTCT TTCATTGCCA
TGTTCCCACT TGTGATAGGG TATAGTGGTC GAGTGGCAGA AAGTAACGGT

CM(R)
3701 TACGGAACTC CGGGTGAGCA TTCATCAGGC GGGCAAGAAT GTGAATAAAG
ATGCCTTGAG GCCCACTCGT AAGTAGTCCG CCCGTTCTTA CACTTATTTC

CM(R)
3751 GCCGGATAAA ACTTGTGCTT ATTTTTCTTT ACGGTCTTTA AAAAGGCCGT
CGGCCTATTT TGAACACGAA TAAAAAGAAA TGCCAGAAAT TTTTCCGGCA

CM(R)
3801 AATATCCAGC TGAACGGTCT GGTTATAGGT ACATTGAGCA ACTGACTGAA
TTATAGGTCG ACTTGCCAGA CCAATATCCA TGTAACTCGT TGACTGACTT

CM(R)
3851 ATGCCTCAAA ATGTTCTTTA CGATGCCATT GGGATATATC AACGGTGGTA
TACGGAGTTT TACAAGAAAT GCTACGGTAA CCCTATATAG TTGCCACCAT

CM(R)
3901 TATCCAGTGA TTTTTTTCTC CATTTTAGCT TCCTTAGCTC CTGAAAATCT
ATAGGTCACT AAAAAAAGAG GTAAAATCGA AGGAATCGAG GACTTTTAGA

CM(R) SD

cat promoter

3951 CGATAACTCA AAAAATACGC CCGGTAGTGA TCTTATTTCA TTATGGTGAA
GCTATTGAGT TTTTTATGCG GGCCATCACT AGAATAAAGT AATACCACTT

cat promoter
CRP site

4001 AGTTGGAACC TCACCCGACG TCTAATGTGA GTTAGCTCAC TCATTAGGCA
TCAACCTTGG AGTGGGCTGC AGATTACACT CAATCGAGTG AGTAATCCGT

cat promoter
lac mRNA start
lac operator
-35 region -10 region

4051 CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATGTTGT GTGGAATTGT
GGGGTCCGAA ATGTGAAATA CGAAGGCCGA GCATACAACA CACCTTAACA
lac operator SD lacZ′

4101 GAGCGGATAA CAATTTCACA CAGGAAACAG CTATGACCAT GATTACGAAT
CTCGCCTATT GTTAAAGTGT GTCCTTTGTC GATACTGGTA CTAATGCTTA
lacZ′

4151 T
A

Figure 30

VL										Framework 1								
Position	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8
	EcoRV						BanII											
VLk1 (SEQ ID NO: 61)	GAT	ATC	CAG	ATG	ACC	CAG	AGC	CCG	TCT	AGC	CTG	AGC	GCG	AGC	GTG	GGT	GAT	CGT
MSPro28 (SEQ ID NO:62)	GAT	ATC	CAG	ATG	ACC	CAG	AGC	CCG	TCT	AGC	CTG	AGC	GCG	AGC	GTG	GGT	GAT	CGT
VLk3 (SEQ ID NO: 63)	GAT	ATC	GTG	CTG	ACC	CAG	AGC	CCG	GCG	ACC	CTG	AGC	CTG	TCT	CCG	GGC	GAA	CGT
MSPro24 (SEQ ID NO:64)	GAT	ATC	GTG	CTG	ACC	CAG	AGC	CCG	GCG	ACC	CTG	AGC	CTG	TCT	CCG	GGC	GAA	CGT
MSPro29 (SEQ ID NO:65)	GAT	ATC	GTG	CTG	ACC	CAG	AGC	CCG	GCG	ACC	CTG	AGC	CTG	TCT	CCG	GGC	GAA	CGT
VLk4 (SEQ ID NO:66)	GAT	ATC	GTG	ATG	ACC	CAG	AGC	CCG	GAT	AGC	CTG	GCG	GTG	AGC	CTG	GGC	GAA	CGT
MSPro21 (SEQ ID NO:67)	GAT	ATC	GTG	ATG	ACC	CAG	AGC	CCG	GAT	AGC	CTG	GCG	GTG	AGC	CTG	GGC	GAA	CGT
VL12 (SEQ ID NO:68)	GAT	ATC	GCA	CTG	ACC	CAG	CCA	GCT	.	TCA	GTG	AGC	GGC	TCA	CCA	GGT	CAG	AGC
MSPro55 (SEQ ID NO:69)	GAT	ATC	GCA	CTG	ACC	CAG	CCA	GCT	.	TCA	GTG	AGC	GGC	TCA	CCA	GGT	CAG	AGC
MSPro11 (SEQ ID NO:70)	GAT	ATC	GCA	CTG	ACC	CAG	CCA	GCT	.	TCA	GTG	AGC	GGC	TCA	CCA	GGT	CAG	AGC
MSPro26 (SEQ ID NO:71)	GAT	ATC	GCA	CTG	ACC	CAG	CCA	GCT	.	TCA	GTG	AGC	GGC	TCA	CCA	GGT	CAG	AGC
VL13 (SEQ ID NO:72)	GAT	ATC	GAA	CTG	ACC	CAG	CCG	CCT	.	TCA	GTG	AGC	GTT	GCA	CCA	GGT	CAG	ACC
MSPro54 (SEQ ID NO: 73)	GAT	ATC	GAA	CTG	ACC	CAG	CCG	CCT	.	TCA	GTG	AGC	GTT	GCA	CCA	GGT	CAG	ACC
MSPro2 (SEQ ID NO:74)	GAT	ATC	GAA	CTG	ACC	CAG	CCG	CCT	.	TCA	GTG	AGC	GTT	GCA	CCA	GGT	CAG	ACC
MSPro12 (SEQ ID NO: 75)	GAT	ATC	GAA	CTG	ACC	CAG	CCG	CCT	.	TCA	GTG	AGC	GTT	GCA	CCA	GGT	CAG	ACC
MSPro59 (SEQ ID NO: 76)	GAT	ATC	GAA	CTG	ACC	CAG	CCG	CCT	.	TCA	GTG	AGC	GTT	GCA	CCA	GGT	CAG	ACC

VH										Framework 1								
Position	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8
			MfeI															
VH1A (SEQ ID NO:77)	GAA/CAG	GTG	CAA	TTG	GTT	CAG	TCT	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	AGC	AGC	GTG
MSPro21 (SEQ ID NO:78)	CAG	GTG	CAA	TTG	GTT	CAG	TCT	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	AGC	AGC	GTG
MSPro24 (SEQ ID NO: 79)	CAG	GTG	CAA	TTG	GTT	CAG	TCT	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	AGC	AGC	GTG
MSPro28 (SEQ ID NO:80)	CAG	GTG	CAA	TTG	GTT	CAG	TCT	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	AGC	AGC	GTG
VH1B (SEQ ID NO: 81)	GAA/CAG	GTG	CAA	TTG	GTT	CAG	AGC	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	GCG	AGC	GTG
MSPro54 (SEQ ID NO:82)	CAG	GTG	CAA	TTG	GTT	CAG	AGC	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	GCG	AGC	GTG
MSPro55 (SEQ ID NO: 83)	CAG	GTG	CAA	TTG	GTT	CAG	AGC	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	GCG	AGC	GTG
MSPro2 (SEQ ID NO:84)	CAG	GTG	CAA	TTG	GTT	CAG	AGC	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	GCG	AGC	GTG
MSPro11 (SEQ ID NO:85)	CAG	GTG	CAA	TTG	GTT	CAG	AGC	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	GCG	AGC	GTG
MSPro26 (SEQ ID NO:86)	CAG	GTG	CAA	TTG	GTT	CAG	AGC	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	GCG	AGC	GTG
MSPro29 (SEQ ID NO:87)	CAG	GTG	CAA	TTG	GTT	CAG	AGC	GGC	GCG	GAA	GTG	AAA	AAA	CCG	GGC	GCG	AGC	GTG
VH2 (SEQ ID NO:88)	GAA/CAG	GTG	CAA	TTG	AAA	GAA	AGC	GGC	CCG	GCC	CTG	GTG	AAA	CCG	ACC	CAA	ACC	CTG
MSPro12 (SEQ ID NO:89)	CAG	GTG	CAA	TTG	AAA	GAA	AGC	GGC	CCG	GCC	CTG	GTG	AAA	CCG	ACC	CAA	ACC	CTG
VH6 (SEQ ID NO:90)	GAA/CAG	GTG	CAA	TTG	CAA	CAG	TCT	GGT	CCG	GGC	CTG	GTG	AAA	CCG	AGC	CAA	ACC	CTG
MSPro59 (SEQ ID NO:91)	CAG	GTG	CAA	TTG	CAA	CAG	TCT	GGT	CCG	GGC	CTG	GTG	AAA	CCG	AGC	CAA	ACC	CTG

CDR 1

PstI KpnI

9	20	1	2	3	4	5	6	7	8	9	30	a	b	c	d	e	f	1	2	3	4	5	6	7	8
GTG	ACC	ATT	ACC	TGC	AGA	GCG	AGC	CAG	GGC	ATT	AGC	AGC	---	---	---	---	---	---	TAT	CTG	GCG	TGG	TAC	CAG	CAG
GTG	ACC	ATT	ACC	TGC	AGA	GCG	AGC	CAG	GGC	ATT	AGC	AGC	---	---	---	---	---	---	TAT	CTG	GCG	TGG	TAC	CAG	CAG
GCG	ACC	CTG	AGC	TGC	AGA	GCG	AGC	CAG	AGC	GTG	AGC	AGC	---	---	---	---	---	AGC	TAT	CTG	GCG	TGG	TAC	CAG	CAG
GCG	ACC	CTG	AGC	TGC	AGA	GCG	AGC	CAG	AGC	GTG	AGC	AGC	---	---	---	---	---	AGC	TAT	CTG	GCG	TGG	TAC	CAG	CAG
GCG	ACC	CTG	AGC	TGC	AGA	GCG	AGC	CAG	AGC	GTG	AGC	AGC	---	---	---	---	---	AGC	TAT	CTG	GCG	TGG	TAC	CAG	CAG
GCG	ACC	ATT	AAC	TGC	AGA	AGC	AGC	CAG	AGC	GTG	CTG	TAT	AGC	AGC	AAC	AAC	AAA	AAC	TAT	CTG	GCG	TGG	TAC	CAG	CAG
GCG	ACC	ATT	AAC	TGC	AGA	AGC	AGC	CAG	AGC	GTG	CTG	TAT	AGC	AGC	AAC	AAC	AAA	AAC	TAT	CTG	GCG	TGG	TAC	CAG	CAG
ATT	ACC	ATC	TCG	TGT	ACG	GGT	ACT	AGC	AGC	GAT	GTG	GGC	GGC	TAT	AAC	---	---	---	TAT	GTG	AGC	TGG	TAC	CAG	CAG
ATT	ACC	ATC	TCG	TGT	ACG	GGT	ACT	AGC	AGC	GAT	GTG	GGC	GGC	TAT	AAC	---	---	---	TAT	GTG	AGC	TGG	TAC	CAG	CAG
ATT	ACC	ATC	TCG	TGT	ACG	GGT	ACT	AGC	AGC	GAT	GTG	GGC	GGC	TAT	AAC	---	---	---	TAT	GTG	AGC	TGG	TAC	CAG	CAG
ATT	ACC	ATC	TCG	TGT	ACG	GGT	ACT	AGC	AGC	GAT	GTG	GGC	GGC	TAT	AAC	---	---	---	TAT	GTG	AGC	TGG	TAC	CAG	CAG
GCG	CGT	ATC	TCG	TGT	AGC	GGC	---	GAT	GCG	---	CTG	GGC	GAT	AAA	---	---	---	---	TAC	GCG	AGC	TGG	TAC	CAG	CAG
GCG	CGT	ATC	TCG	TGT	AGC	GGC	---	GAT	GCG	---	CTG	GGC	GAT	AAA	---	---	---	---	TAC	GCG	AGC	TGG	TAC	CAG	CAG
GCG	CGT	ATC	TCG	TGT	AGC	GGC	---	GAT	GCG	---	CTG	GGC	GAT	AAA	---	---	---	---	TAC	GCG	AGC	TGG	TAC	CAG	CAG
GCG	CGT	ATC	TCG	TGT	AGC	GGC	---	GAT	GCG	---	CTG	GGC	GAT	AAA	---	---	---	---	TAC	GCG	AGC	TGG	TAC	CAG	CAG
GCG	CGT	ATC	TCG	TGT	AGC	GGC	---	GAT	GCG	---	CTG	GGC	GAT	AAA	---	---	---	---	TAC	GCG	AGC	TGG	TAC	CAG	CAG

CDR 1 Framew

BspEI BstXI

9	20	1	2	3	4	5	6	7	8	9	30	1	a	b	2	3	4	5	6	7	8	9	40	1	2
AAA	GTG	AGC	TGC	AAA	GCC	TCC	GGA	GGC	ACT	TTT	AGC	AGC	-	-	TAT	GCG	ATT	AGC	TGG	GTG	CGC	CAA	GCC	CCT	GGG
AAA	GTG	AGC	TGC	AAA	GCC	TCC	GGA	GGC	ACT	TTT	AGC	AGC	-	-	TAT	GCG	ATT	AGC	TGG	GTG	CGC	CAA	GCC	CCT	GGG
AAA	GTG	AGC	TGC	AAA	GCC	TCC	GGA	GGC	ACT	TTT	AGC	AGC	-	-	TAT	GCG	ATT	AGC	TGG	GTG	CGC	CAA	GCC	CCT	GGG
AAA	GTG	AGC	TGC	AAA	GCC	TCC	GGA	GGC	ACT	TTT	AGC	AGC	-	-	TAT	GCG	ATT	AGC	TGG	GTG	CGC	CAA	GCC	CCT	GGG
AAA	GTG	AGC	TGC	AAA	GCC	TCC	GGA	TAT	ACC	TTT	ACC	AGC	-	-	TAT	TAT	ATG	CAC	TGG	GTC	CGC	CAA	GCC	CCT	GGG
AAA	GTG	AGC	TGC	AAA	GCC	TCC	GGA	TAT	ACC	TTT	ACC	AGC	-	-	TAT	TAT	ATG	CAC	TGG	GTC	CGC	CAA	GCC	CCT	GGG
AAA	GTG	AGC	TGC	AAA	GCC	TCC	GGA	TAT	ACC	TTT	ACC	AGC	-	-	TAT	TAT	ATG	CAC	TGG	GTC	CGC	CAA	GCC	CCT	GGG
AAA	GTG	AGC	TGC	AAA	GCC	TCC	GGA	TAT	ACC	TTT	ACC	AGC	-	-	TAT	TAT	ATG	CAC	TGG	GTC	CGC	CAA	GCC	CCT	GGG
AAA	GTG	AGC	TGC	AAA	GCC	TCC	GGA	TAT	ACC	TTT	ACC	AGC	-	-	TAT	TAT	ATG	CAC	TGG	GTC	CGC	CAA	GCC	CCT	GGG
AAA	GTG	AGC	TGC	AAA	GCC	TCC	GGA	TAT	ACC	TTT	ACC	AGC	-	-	TAT	TAT	ATG	CAC	TGG	GTC	CGC	CAA	GCC	CCT	GGG
AAA	GTG	AGC	TGC	AAA	GCC	TCC	GGA	TAT	ACC	TTT	ACC	AGC	-	-	TAT	TAT	ATG	CAC	TGG	GTC	CGC	CAA	GCC	CCT	GGG
ACC	CTG	ACC	TGT	ACC	TTT	TCC	GGA	TTT	AGC	CTG	TCC	ACG	TCT	GGC	GTT	GGC	GTG	GGC	TGG	ATT	CGC	CAG	CCG	CCT	GGG
ACC	CTG	ACC	TGT	ACC	TTT	TCC	GGA	TTT	AGC	CTG	TCC	ACG	TCT	GGC	GTT	GGC	GTG	GGC	TGG	ATT	CGC	CAG	CCG	CCT	GGG
AGC	CTG	ACC	TGT	GCG	ATT	TCC	GGA	GAT	AGC	GTG	AGC	AGC	AAC	AGC	GCG	GCG	TGG	AAC	TGG	ATT	CGC	CAG	TCT	CCT	GGG
AGC	CTG	ACC	TGT	GCG	ATT	TCC	GGA	GAT	AGC	GTG	AGC	AGC	AAC	AGC	GCG	GCG	TGG	AAC	TGG	ATT	CGC	CAG	TCT	CCT	GGG

Framework 2											CDR 2														
	4										5										6				
9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4
	SexAI						AseI											SanDI							
AAA	CCA	GGT	AAA	GCA	CCG	AAA	CTA	TTA	ATT	TAT	GCA	GCC	AGC	AGC	TTG	CAA	AGC	GGG	GTC	CCG	TCC	CGT	TTT	AGC	GGC
AAA	CCA	GGT	AAA	GCA	CCG	AAA	CTA	TTA	ATT	TAT	GCA	GCC	AGC	AGC	TTG	CAA	AGC	GGG	GTC	CCG	TCC	CGT	TTT	AGC	GGC
AAA	CCA	GGT	CAA	GCA	CCG	CGT	CTA	TTA	ATT	TAT	GGC	GCG	AGC	AGC	CGT	GCA	ACT	GGG	GTC	CCG	GCG	CGT	TTT	AGC	GGC
AAA	CCA	GGT	CAA	GCA	CCG	CGT	CTA	TTA	ATT	TAT	GGC	GCG	AGC	AGC	CGT	GCA	ACT	GGG	GTC	CCG	GCG	CGT	TTT	AGC	GGC
AAA	CCA	GGT	CAA	GCA	CCG	CGT	CTA	TTA	ATT	TAT	GGC	GCG	AGC	AGC	CGT	GCA	ACT	GGG	GTC	CCG	GCG	CGT	TTT	AGC	GGC
AAA	CCA	GGT	CAG	CCG	CCG	AAA	CTA	TTA	ATT	TAT	TGG	GCA	TCC	ACC	CGT	GAA	AGC	GGG	GTC	CCG	GAT	CGT	TTT	AGC	GGC
AAA	CCA	GGT	CAG	CCG	CCG	AAA	CTA	TTA	ATT	TAT	TGG	GCA	TCC	ACC	CGT	GAA	AGC	GGG	GTC	CCG	GAT	CGT	TTT	AGC	GGC
CAT	CCC	GGG	AAG	GCG	CCG	AAA	CTG	ATG	ATT	TAT	GAT	GTG	AGC	AAC	CGT	CCC	TCA	GGC	GTG	AGC	AAC	CGT	TTT	AGC	GGA
CAT	CCC	GGG	AAG	GCG	CCG	AAA	CTG	ATG	ATT	TAT	GAT	GTG	AGC	AAC	CGT	CCC	TCA	GGC	GTG	AGC	AAC	CGT	TTT	AGC	GGA
CAT	CCC	GGG	AAG	GCG	CCG	AAA	CTG	ATG	ATT	TAT	GAT	GTG	AGC	AAC	CGT	CCC	TCA	GGC	GTG	AGC	AAC	CGT	TTT	AGC	GGA
CAT	CCC	GGG	AAG	GCG	CCG	AAA	CTG	ATG	ATT	TAT	GAT	GTG	AGC	AAC	CGT	CCC	TCA	GGC	GTG	AGC	AAC	CGT	TTT	AGC	GGA
AAA	CCC	GGG	CAG	GCG	CCA	GTT	CTG	GTG	ATT	TAT	GAT	GAT	TCT	GAC	CGT	CCC	TCA	GGC	ATC	CCG	GAA	CGC	TTT	AGC	GGA
AAA	CCC	GGG	CAG	GCG	CCA	GTT	CTG	GTG	ATT	TAT	GAT	GAT	TCT	GAC	CGT	CCC	TCA	GGC	ATC	CCG	GAA	CGC	TTT	AGC	GGA
AAA	CCC	GGG	CAG	GCG	CCA	GTT	CTG	GTG	ATT	TAT	GAT	GAT	TCT	GAC	CGT	CCC	TCA	GGC	ATC	CCG	GAA	CGC	TTT	AGC	GGA
AAA	CCC	GGG	CAG	GCG	CCA	GTT	CTG	GTG	ATT	TAT	GAT	GAT	TCT	GAC	CGT	CCC	TCA	GGC	ATC	CCG	GAA	CGC	TTT	AGC	GGA
AAA	CCC	GGG	CAG	GCG	CCA	GTT	CTG	GTG	ATT	TAT	GAT	GAT	TCT	GAC	CGT	CCC	TCA	GGC	ATC	CCG	GAA	CGC	TTT	AGC	GGA

work 2							CDR 2																		
							5													6					
3	4	5	6	7	8	9	0	1	2	a	b	c	3	4	5	6	7	8	9	0	1	2	3	4	5
		XhoI																							
CAG	GGT	CTC	GAG	TGG	ATG	GGC	GGC	ATT	ATT	CCG	-	-	ATT	TTT	GGC	ACG	GCG	AAC	TAC	GCG	CAG	AAG	TTT	CAG	GGC
CAG	GGT	CTC	GAG	TGG	ATG	GGC	GGC	ATT	ATT	CCG	-	-	ATT	TTT	GGC	ACG	GCG	AAC	TAC	GCG	CAG	AAG	TTT	CAG	GGC
CAG	GGT	CTC	GAG	TGG	ATG	GGC	GGC	ATT	ATT	CCG	-	-	ATT	TTT	GGC	ACG	GCG	AAC	TAC	GCG	CAG	AAG	TTT	CAG	GGC
CAG	GGT	CTC	GAG	TGG	ATG	GGC	GGC	ATT	ATT	CCG	-	-	ATT	TTT	GGC	ACG	GCG	AAC	TAC	GCG	CAG	AAG	TTT	CAG	GGC
CAG	GGT	CTC	GAG	TGG	ATG	GGC	TGG	ATT	AAC	CCG	-	-	AAT	AGC	GGC	GGC	ACG	AAC	TAC	GCG	CAG	AAG	TTT	CAG	GGC
CAG	GGT	CTC	GAG	TGG	ATG	GGC	TGG	ATT	AAC	CCG	-	-	AAT	AGC	GGC	GGC	ACG	AAC	TAC	GCG	CAG	AAG	TTT	CAG	GGC
CAG	GGT	CTC	GAG	TGG	ATG	GGC	TGG	ATT	AAC	CCG	-	-	AAT	AGC	GGC	GGC	ACG	AAC	TAC	GCG	CAG	AAG	TTT	CAG	GGC
CAG	GGT	CTC	GAG	TGG	ATG	GGC	TGG	ATT	AAC	CCG	-	-	AAT	AGC	GGC	GGC	ACG	AAC	TAC	GCG	CAG	AAG	TTT	CAG	GGC
CAG	GGT	CTC	GAG	TGG	ATG	GGC	TGG	ATT	AAC	CCG	-	-	AAT	AGC	GGC	GGC	ACG	AAC	TAC	GCG	CAG	AAG	TTT	CAG	GGC
CAG	GGT	CTC	GAG	TGG	ATG	GGC	TGG	ATT	AAC	CCG	-	-	AAT	AGC	GGC	GGC	ACG	AAC	TAC	GCG	CAG	AAG	TTT	CAG	GGC
CAG	GGT	CTC	GAG	TGG	ATG	GGC	TGG	ATT	AAC	CCG	-	-	AAT	AGC	GGC	GGC	ACG	AAC	TAC	GCG	CAG	AAG	TTT	CAG	GGC
AAA	GCC	CTC	GAG	TGG	CTG	GCT	CTG	ATT	GAT		-	-	TGG	GAT	GAT	GAT	AAG	TAT	TAT	AGC	ACC	AGC	CTG	AAA	ACG
AAA	GCC	CTC	GAG	TGG	CTG	GCT	CTG	ATT	GAT		-	-	TGG	GAT	GAT	GAT	AAG	TAT	TAT	AGC	ACC	AGC	CTG	AAA	ACG
CGT	GGC	CTC	GAG	TGG	CTG	GGC	CGT	ACC	TAT	TAT	CGT	-	AGC	AAA	TGG	TAT	AAC	GAT	TAT	GCG	GTG	AGC	GTG	AAA	AGC
CGT	GGC	CTC	GAG	TGG	CTG	GGC	CGT	ACC	TAT	TAT	CGT	-	AGC	AAA	TGG	TAT	AAC	GAT	TAT	GCG	GTG	AGC	GTG	AAA	AGC

Framework 3																							
					7										8								
5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8
	BamHI															BbsI							
TCT	GGA	TCC	GGC	ACT	GAT	TTT	ACC	CTG	ACC	ATT	AGC	AGC	CTG	CAA	CCT	GAA	GAC	TTT	GCG	T/G	TAT	TAT	TGC
TCT	GGA	TCC	GGC	ACT	GAT	TTT	ACC	CTG	ACC	ATT	AGC	AGC	CTG	CAA	CCT	GAA	GAC	TTT	GCG	GTT	TAT	TAT	TGC
TCT	GGA	TCC	GGC	ACG	GAT	TTT	ACC	CTG	ACC	ATT	AGC	AGC	CTG	GAA	CCT	GAA	GAC	TTT	GCG	T/G	TAT	TAT	TGC
TCT	GGA	TCC	GGC	ACG	GAT	TTT	ACC	CTG	ACC	ATT	AGC	AGC	CTG	GAA	CCT	GAA	GAC	TTT	GCG	ACT	TAT	TAT	TGC
TCT	GGA	TCC	GGC	ACG	GAT	TTT	ACC	CTG	ACC	ATT	AGC	AGC	CTG	GAA	CCT	GAA	GAC	TTT	GCG	ACT	TAT	TAT	TGC
TCT	GGA	TCC	GGC	ACT	GAT	TTT	ACC	CTG	ACC	ATT	TCG	TCC	CTG	CAA	GCT	GAA	GAC	GTG	GCG	GTG	TAT	TAT	TGC
TCT	GGA	TCC	GGC	ACT	GAT	TTT	ACC	CTG	ACC	ATT	TCG	TCC	CTG	CAA	GCT	GAA	GAC	GTG	GCG	GTG	TAT	TAT	TGC
TCC	AAA	AGC	GGC	AAC	ACC	GCG	AGC	CTG	ACC	ATT	AGC	GGC	CTG	CAA	GCG	GAA	GAC	GAA	GCG	GAT	TAT	TAT	TGC
TCC	AAA	AGC	GGC	AAC	ACC	GCG	AGC	CTG	ACC	ATT	AGC	GGC	CTG	CAA	GCG	GAA	GAC	GAA	GCG	GAT	TAT	TAT	TGC
TCC	AAA	AGC	GGC	AAC	ACC	GCG	AGC	CTG	ACC	ATT	AGC	GGC	CTG	CAA	GCG	GAA	GAC	GAA	GCG	GAT	TAT	TAT	TGC
TCC	AAA	AGC	GGC	AAC	ACC	GCG	AGC	CTG	ACC	ATT	AGC	GGC	CTG	CAA	GCG	GAA	GAC	GAA	GCG	GAT	TAT	TAT	TGC
TCC	AAC	AGC	GGC	AAC	ACC	GCG	ACC	CTG	ACC	ATT	AGC	GGC	ACT	CAG	GCG	GAA	GAC	GAA	GCG	GAT	TAT	TAT	TGC
TCC	AAC	AGC	GGC	AAC	ACC	GCG	ACC	CTG	ACC	ATT	AGC	GGC	ACT	CAG	GCG	GAA	GAC	GAA	GCG	GAT	TAT	TAT	TGC
TCC	AAC	AGC	GGC	AAC	ACC	GCG	ACC	CTG	ACC	ATT	AGC	GGC	ACT	CAG	GCG	GAA	GAC	GAA	GCG	GAT	TAT	TAT	TGC
TCC	AAC	AGC	GGC	AAC	ACC	GCG	ACC	CTG	ACC	ATT	AGC	GGC	ACT	CAG	GCG	GAA	GAC	GAA	GCG	GAT	TAT	TAT	TGC
TCC	AAC	AGC	GGC	AAC	ACC	GCG	ACC	CTG	ACC	ATT	AGC	GGC	ACT	CAG	GCG	GAA	GAC	GAA	GCG	GAT	TAT	TAT	TGC

Framework 3																							
				7										8									
6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	a	b	c	3	4	5	6
	BstEII							NspV															
CGG	GTG	ACC	ATT	ACC	GCG	GAT	GAA	AGC	ACC	AGC	ACC	GCG	TAT	ATG	GAA	CTG	AGC	AGC	CTG	CGT	AGC	GAA	GAT
CGG	GTG	ACC	ATT	ACC	GCG	GAT	GAA	AGC	ACC	AGC	ACC	GCG	TAT	ATG	GAA	CTG	AGC	AGC	CTG	CGT	AGC	GAA	GAT
CGG	GTG	ACC	ATT	ACC	GCG	GAT	GAA	AGC	ACC	AGC	ACC	GCG	TAT	ATG	GAA	CTG	AGC	AGC	CTG	CGT	AGC	GAA	GAT
CGG	GTG	ACC	ATT	ACC	GCG	GAT	GAA	AGC	ACC	AGC	ACC	GCG	TAT	ATG	GAA	CTG	AGC	AGC	CTG	CGT	AGC	GAA	GAT
CGG	GTG	ACC	ATG	ACC	CGT	GAT	ACC	AGC	ATT	AGC	ACC	GCG	TAT	ATG	GAA	CTG	AGC	AGC	CTG	CGT	AGC	GAA	GAT
CGG	GTG	ACC	ATG	ACC	CGT	GAT	ACC	AGC	ATT	AGC	ACC	GCG	TAT	ATG	GAA	CTG	AGC	AGC	CTG	CGT	AGC	GAA	GAT
CGG	GTG	ACC	ATG	ACC	CGT	GAT	ACC	AGC	ATT	AGC	ACC	GCG	TAT	ATG	GAA	CTG	AGC	AGC	CTG	CGT	AGC	GAA	GAT
CGG	GTG	ACC	ATG	ACC	CGT	GAT	ACC	AGC	ATT	AGC	ACC	GCG	TAT	ATG	GAA	CTG	AGC	AGC	CTG	CGT	AGC	GAA	GAT
CGG	GTG	ACC	ATG	ACC	CGT	GAT	ACC	AGC	ATT	AGC	ACC	GCG	TAT	ATG	GAA	CTG	AGC	AGC	CTG	CGT	AGC	GAA	GAT
CGG	GTG	ACC	ATG	ACC	CGT	GAT	ACC	AGC	ATT	AGC	ACC	GCG	TAT	ATG	GAA	CTG	AGC	AGC	CTG	CGT	AGC	GAA	GAT
CGG	GTG	ACC	ATG	ACC	CGT	GAT	ACC	AGC	ATT	AGC	ACC	GCG	TAT	ATG	GAA	CTG	AGC	AGC	CTG	CGT	AGC	GAA	GAT
CGT	CTG	ACC	ATT	AGC	AAA	GAT	ACT	TCG	AAA	AAT	CAG	GTG	GTG	CTG	ACT	ATG	ACC	AAC	ATG	GAC	CCG	GTG	GAT
CGT	CTG	ACC	ATT	AGC	AAA	GAT	ACT	TCG	AAA	AAT	CAG	GTG	GTG	CTG	ACT	ATG	ACC	AAC	ATG	GAC	CCG	GTG	GAT
CGG	ATT	ACC	ATC	AAC	CCG	GAT	ACT	TCG	AAA	AAC	CAG	TTT	AGC	CTG	CAA	CTG	AAC	AGC	GTG	ACC	CCG	GAA	GAT
CGG	ATT	ACC	ATC	AAC	CCG	GAT	ACT	TCG	AAA	AAC	CAG	TTT	AGC	CTG	CAA	CTG	AAC	AGC	GTG	ACC	CCG	GAA	GAT

CDR 3														Framework 4							
	9												10								
9	0	1	2	3	4	5	a	b	6	7	8	9	0	1	2	3	4	5	6	7	8
											MscI										BsiWI
x	CAG	x	x	x	x	x			x	ACC	TTT	GGC	CAG	GGT	ACG	AAA	GTT	GAA	ATT	AAA	CGT	ACG
TTT	CAG	TAT	GGT	TCT	ATT	CCT	CCT			ACC	TTT	GGC	CAG	GGT	ACG	AAA	GTT	GAA	ATT	AAA	CGT	ACG
x	CAG	x	x	x	x	x			x	ACC	TTT	GGC	CAG	GGT	ACG	AAA	GTT	GAA	ATT	AAA	CGT	ACG
CAG	CAG	ATG	TCT	AAT	TAT	CCT	GAT			ACC	TTT	GGC	CAG	GGT	ACG	AAA	GTT	GAA	ATT	AAA	CGT	ACG
CAG	CAG	ACT	AAT	AAT	GCT	CCT	GTT			ACC	TTT	GGC	CAG	GGT	ACG	AAA	GTT	GAA	ATT	AAA	CGT	ACG
x	CAG	x	x	x	x	x			x	ACC	TTT	GGC	CAG	GGT	ACG	AAA	GTT	GAA	ATT	AAA	CGT	ACG
CAG	CAG	TAT	GAT	TCT	ATT	CCT	TAT			ACC	TTT	GGC	CAG	GGT	ACG	AAA	GTT	GAA	ATT	AAA	CGT	ACG
CAG	x	x	GAC	x	x	x	(x)	(x)	x	GTG	TTT	GGC	GGC	GGC	ACG	AAG	TTA	ACC	GTT	CTT	GGC	CAG
CAG	AGC	TAT	GAC	ATG	TAT	AAT	TAT	ATT		GTG	TTT	GGC	GGC	GGC	ACG	AAG	TTA	ACC	GTT	CTT	GGC	CAG
CAG	TCT	CAT		CAT	TTT	TAT	GAG			GTG	TTT	GGC	GGC	GGC	ACG	AAG	TTA	ACC	GTT	CTT	GGC	CAG
CAG	AGC	TAT	GAC	AAT	AAT	TCT	GAT	GTT		GTG	TTT	GGC	GGC	GGC	ACG	AAG	TTA	ACC	GTT	CTT	GGC	CAG
CAG	x	x	GAC	x	x	x	(x)	(x)	x	GTG	TTT	GGC	GGC	GGC	ACG	AAG	TTA	ACC	GTT	CTT	GGC	CAG
CAG	AGC	TAT	GAC	TAT	TTT	AAG	CTT	x	x	GTG	TTT	GGC	GGC	GGC	ACG	AAG	TTA	ACC	GTT	CTT	GGC	CAG
CAG	AGC	TAT	GAC	TAT	TCT	GCT	GAT	TAT		GTG	TTT	GGC	GGC	GGC	ACG	AAG	TTA	ACC	GTT	CTT	GGC	CAG
CAG	AGC	TAT	GAC	TTT	GAT	TTT	GCT			GTG	TTT	GGC	GGC	GGC	ACG	AAG	TTA	ACC	GTT	CTT	GGC	CAG
CAG	AGC	TAT	GAC	GGT	CCT	GAT	CTT	TGG		GTG	TTT	GGC	GGC	GGC	ACG	AAG	TTA	ACC	GTT	CTT	GGC	CAG

								CDR 3														
			9										10									
7	8	9	0	1	2	3	4	5	6	7	8	9	0	a	b	c	d	e	f	g	h	i
	EagI				BssHII																	
ACG	GCC	GTG	TAT	TAT	TGC	GCG	CGT	x	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	x
ACG	GCC	GTG	TAT	TAT	TGC	GCG	CGT	x	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	GAT	AAT	TGG	TTT	AAG
ACG	GCC	GTG	TAT	TAT	TGC	GCG	CGT	x	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	GTT	AAT	CAT	TGG	ACT
ACG	GCC	GTG	TAT	TAT	TGC	GCG	CGT	x	(x)	(x)	(x)	(x)	GGT	GGT	GGT	TGG	GTT	TCT	CAT	GGT	TAT	TAT
ACG	GCC	GTG	TAT	TAT	TGC	GCG	CGT	x	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	x
ACG	GCC	GTG	TAT	TAT	TGC	GCG	CGT	x	(x)	(x)	AAT	ATG	GCT	TAT	ACT	AAT	TAT	CAG	TAT	GTT	AAT	ATG
ACG	GCC	GTG	TAT	TAT	TGC	GCG	CGT	x	(x)	(x)	TCT	ATG	AAT	TCT	ACT	ATG	TAT	TGG	TAT	CTT	CGT	CGT
ACG	GCC	GTG	TAT	TAT	TGC	GCG	CGT	x	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	GAT	TTT	CTT	GGT
ACG	GCC	GTG	TAT	TAT	TGC	GCG	CGT	x	(x)	(x)	TAT	TAT	GGT	TCT	TCT	CTT	TAT	CAT	TAT	GTT	TTT	GGT
ACG	GCC	GTG	TAT	TAT	TGC	GCG	CGT	x	(x)	(x)	GGT	TAT	TGG	TAT	GCT	TAT	TTT	ACT	TAT	ATT	AAT	TAT
ACG	GCC	GTG	TAT	TAT	TGC	GCG	CGT	x	(x)	ACT	TGG	CAG	TAT	TCT	TAT	TTT	TAT	TAT	CTT	GAT	GGT	GGT
ACG	GCC	ACC	TAT	TAT	TGC	GCG	CGT	x	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	x
ACG	GCC	ACC	TAT	TAT	TGC	GCG	CGT	TAT	CAT	TCT	TGG	TAT	GAG	ATG	GGT	TAT	TAT	GGT	TCT	ACT	GTT	GGT
ACG	GCC	GTG	TAT	TAT	TGC	GCG	CGT	x	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	x
ACG	GCC	GTG	TAT	TAT	TGC	GCG	CGT	x	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	TCT	TAT	TAT

					Framework 4											
j	k	l	1	2	3	4	5	6	7	8	9	110	1	2	3	
							StyI							BlpI		
x	x	x	GAT	x	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
CCT	TTT	TCT	GAT	GTT	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
TAT	ACT	TTT	GAT	TAT	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
TAT	CTT	TTT	GAT	CTT	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
x	x	x	GAT	x	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
CCT	CAT	TTT	GAT	TAT	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
GTT	CTT	TTT	GAT	CAT	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
TAT	GAG	TTT	GAT	TAT	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
GGT	TTT	ATT	GAT	TAT	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
GGT	TAT	TTT	GAT	AAT	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
TAT	TAT	TTT	GAT	ATT	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
x	x	x	GAT	x	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
TAT	ATG	TTT	GAT	TAT	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
x	x	x	GAT	x	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC
CCT	GAT	TTT	GAT	TAT	TGG	GGC	CAA	GGC	ACC	CTG	GTG	ACG	GTT	AGC	TCA	GC

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 December 2002 (27.12.2002)

PCT

(10) International Publication Number
WO 2002/102973 A3

(51) International Patent Classification[7]: C07K 16/00, A61K 39/395

(21) International Application Number: PCT/IL2002/000495

(22) International Filing Date: 20 June 2002 (20.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/299,187 20 June 2001 (20.06.2001) US

(71) Applicant (*for all designated States except US*): **PRO-CHON BIOTECH LTD.** [IL/IL]; P.O. Box 1482, 76114 Rehovot (IL).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **YAYON, Avner** [IL/IL]; Moshav Sitria #104, 76834 (IL). **ROM, Eran** [IL/IL]; Gordon Street 43, 76287 Rehovot (IL).

(74) Agent: WEBB, Cynthia; Webb & Associates, P.O. Box 2189, 76121 Rehovot (IL).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report: 26 February 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ANTIBODIES THAT BLOCK RECEPTOR PROTEIN TYROSINE KINASE ACTIVATION, METHODS OF SCREENING FOR AND USES THEREOF

(57) Abstract: Molecules containing the antigen-binding portion of antibodies that block constitutive and/or ligand-dependent activation of a receptor protein tyrosine kinase, such as fibroblast growth factor receptor 3 (FGFR3), are found through screening methods, where a soluble dimeric form of a receptor protein tyrosine kinase is used as target for screening a library of antibody fragments displayed on the surface of bacteriophage. The molecules of the present invention which block constitutive activation can be administered to treat or inhibit skeletal dysplasia, craniosynostosis disorders, cell proliferative diseases or disorders, or tumor progression associated with the constitutive activation of a receptor protein tyrosine kinase.

WO 2002/102973 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL02/00495

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C07K 16/00; A61K 39/395
US CL : 530/387.1, 388.1, 388.22, 388.8, 388.85, 300, 350; 424/130.1, 143.1, 155.1, 156.1.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/387.1, 388.1, 388.22, 388.8, 388.85, 300, 350; 424/130.1, 143.1, 155.1, 156.1.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 6,214,974 B1 (ROSENBLUM et al.) 10 April 2001 (10.04.2001), see entire document, especially column 2, line 44-55.	1, 4-5, 7-8, 11 ----------- 12
Y	US 5,707,632 A (WILLIAMS et al.) 13 January 1998 (13.01.1998), see entire document, especially column 21-23.	1-5, 7-9, 11, 12
X --- Y	US 6,165,464 A (HUDZIAK et al.) 26 December 2000 (26.12.2000), see entire document, especially abstract.	1, 4, 7 ----------- 12
X --- Y	US 6,129,915 A (WELS et al.) 10 October 2000 (10.10.2000), see entire document, especially abstract, column 2, lines 1-5.	1-2, 4, 7 ----------- 12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 18 August 2003 (18.08.2003)	Date of mailing of the international search report 05 DEC 2003
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230	Authorized officer Larry R. Helms Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL02/00495

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claim Nos.: 13-37, 41-85
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-12

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-12, drawn to an antibody.

Group II, claim(s) 38-40, drawn to a method of screening a molecule.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The technical feature linking Groups I-II appears to be an antibody to FGFR. However, Rosenblum et al (US Patent 6,214,974, issued 4/2001) teach such an antibody (see column 2, lines 44-55). Therefore, the technical feature linking the inventions of Groups I-II does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

Continuation of B. FIELDS SEARCHED Item 3:

CAPLUS MEDLINE BIOSIS, WEST, USPATFUL

search terms: FGFR, FGFR3, her2/neu, EGFR, fibroblast growth factor receptor, receptor protein tyrosine kinase, activation.